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Developmental Trade-Offs and Resource Allocation In Caddis Flies.

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A thesis submitted for the degree of Doctor of Philosophy to the Faculty of Science, University of Glasgow.

September 2000

Declaration

I declare that the work presented in this thesis has been completed by myself unless otherwise acknowledged in the text. No part of this work has been presented for any other qualification.

David Stevens

September 2000

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ABSTRACT

Resource allocation and life history strategy are obviously interconnected for all organisms. Limited resources within individuals lead to allocation trade-offs between fitness traits, and constrain their evolution. Many trade-offs have been characterised, mainly concerning the costs of reproduction. Trade-offs occurring between body parts during the growth and development of organisms have received little attention. Such 'Developmental Trade-Offs' between body structures are predicted to have profound effects on an organism's subsequent life history traits. These effects will be particularly significant for organisms where part of the life cycle is spent in a non-feeding form. In such complex life cycles, a complete reorganisation of the body form occurs, often associated with a dramatic change of habitat. Resources accrued in the feeding life history stages are used to build the body and fuel the activities of the non-feeding form. Various factors can lead to variation in the resources available at body reorganisation, which will have a cascade effect into the next life history stage, through the action of developmental trade-offs and strategic allocation

Caddis flies (Order Trichoptera) are one such example of an organism with a complex life cycle. They are holometabolous insects, which means that they undergo a complete metamorphosis, where the aquatic larval form is completely broken down and reconstructed into a terrestrial adult during pupation. Nutrient deficiencies at pupation are predicted to lead to developmental trade-offs, particularly between somatic and reproductive investment. This thesis is an examination of developmental trade-offs occurring in the pupal stage of caddis flies, using both an experimental and correlational approach. Resources available at metamorphosis were manipulated by forcing caddis fly larvae, *Odontocerum albicorne*, to divert more silk, and hence protein, into their larval cases. The resultant adult forms had shorter wings and lighter thoraces (somatic investment) than control individuals, but abdomen mass (reproductive investment) was not affected. The results of this experiment could be due to mechanistic constraints of the developmental pathways during pupation, because resources from the breakdown of certain structures may be 'earmarked' for incorporation into specific adult structures. Equally, the observed response could

represent a strategic preservation of reproductive reserves. *O. albicorne* is a short lived species which mates soon after adult emergence and so sacrificing somatic invetsment may have less drastic affects on fitness.

To examine whether resource allocation pathways during development of the adult form are flexible in caddis flies, the same resource depletion experiment was performed on the caddis fly species, *Glyphotaelius pellucidus*. Adult life span is much longer in this species, which is an adaptation to loss of larval habitat for many months of the year. Here, somatic investment would be predicted to be of much greater importance in the life history, and so if the response to the nutrient deficiencies was strategic, somatic reserves would be expected to be preserved at the expense of reproductive potential. The pattern of resource allocation in the resultant adults was in line with predicted life history requirements, as abdomen mass was sacrificed so as to maintain thoracic mass. Wing length was again shorter, suggesting these patterns were not as a result of strategically maintaining flight ability. This study shows that allocation patterns are not fixed, and that differential response to depletion are possible.

Life history strategy is poorly known for adult caddis flies, particularly those from temporary habitats. An investigation was undertaken to catalogue the Limnephilid species collected over a three-year period from a light trap on Loch Lomondside, and to document their flight periods. In total, 1376 individuals from eleven genera were collected. A range of species were recovered between March and December, and species emerged from a variety of temporary and permanent freshwater larval habitats. Flight periods indicated that life spans ranged from days to months. Eleven of the twenty species caught had not been documented in the area prior to this study. A few species are only commonly found in Scotland, and their biology is relatively poorly studied. Many of the flight periods differed from those published in the literature as typical for these species.

Adults from the first year of the light trap study were used to measure resource dynamics within the adult body. Despite the fact that in animals with complex life cycles many non-feeding stages do not grow, they may show some evidence of 'phenotypic flexibility', and are able to reallocate some body resources to other functions during the life span. Evidence from butterflies suggests individuals

can break down flight muscle mass and use the nitrogen this liberates in reproduction. Patterns of thoracic mass change over time (= age) for the males of seven species (and for females from three of these) from the light trap indicated the same may occur in caddis flies. Evidence from one species reared in captivity, *Limnephilus rhombicus*, demonstrates nitrogen mass declining in the thorax is associated with an increase in nitrogen within the abdomen.

Environmental conditions during the juvenile stage of many univoltine arthropods are known to affect overall adult body size. How this affects resource allocation patterns is however poorly known. Changes in the body size (wing length) and resource allocation (abdomen fat content) of adults emerging over the season was measured for two short lived species of caddis flies, *Lepidostoma hirtum* and *Plectronemia conspersa*. Only *L. hirtum* showed a decline in size, which is probably associated with increasing water temperature in the larval habitat. Changing food conditions were probably responsible for the increase in proportional fat content over the season in *L. hirtum*. *P. conspersa* has a different larval ecology, and seems unaffected by such conditions.

Adult caddis fly life histories are poorly known due to the difficulty of studying the adult stages. The equivalent knowledge of the sister lineage, the butterflies, is much better understood, as is its relationship with adult resource allocation patterns and the relative sizes of males and females. The resource allocation and sexual size dimorphism patterns of 19 species of Limnephilid caddis flies were analysed in an attempt to make predictions concerning mating systems and flight ability. Using theories based on work with butterflies, species were classified on a scale of polyandry to monandry, and as either strong or weak fliers.

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Chapter 1. General Introduction.

Resource allocation and life history strategy are inevitably interconnected for all organisms. The occurrence of long-lived, high fecundity 'Darwinian demons' is precluded by resource allocation trade-offs (Shea *et al.* 1994). Stearns (1992) describes such trade-offs as being "caused by allocation decisions between two or more processes that compete directly with one another for resources within a single individual". This competition for resources occurs because the resources available to an organism, either in its environment or within itself, are limited (Williams 1966). These finite resources must be allocated between a number of life history traits (Fig.1.1.), and increased allocation to one trait will inevitably lead to a decrease in allocation to the other competing traits, known as the 'The Principle of Allocation' (Cody 1966, Levins 1968). These negative associations between traits constrain their evolution (Roff 1992, Stearns 1992) because if trade-offs did not exist then all fitness characters would be maximised within the limits of history and design, and this is not the case (Stearns 1989).

Negative genetic correlations between traits are thought to be the basis of trade-offs (Reznick 1985), operating through the effects of linkage or pleiotropy (Falconer & MacKay 1996). These negative genetic correlations underlie physiological mechanisms that have opposing effects on fitness related traits (Sinervo & Svennson 1998). In the last 15 years, there has been much debate in the literature as to the best way to measure trade-offs (e.g. Partridge & Harvey 1985, 1988; Reznick 1985,1992; Pease and Bull 1988; Bailey 1992; Chippendale *et al.* 1993; O'Shea *et al.* 1994; Rose & Bradley 1998). Direct correlations between fitness traits often give positive rather than negative relationships, not necessarily because of the absence of a trade-off between them, but because of variation between organisms in the resources available to them (e.g. van Noordwijk & de Jong 1986, Lessells 1991, Stearns 1992, Roff 1992). Direct genetic ('selective') or physiological ('mechanistic') manipulations are therefore generally agreed to be the most useful tools for discovering negative associations between traits, but are very difficult to implement, and there are a number of reasons for this (e.g. restricted to a few model organisms, huge sample sizes needed). Therefore, some sort of 'phenotypic'

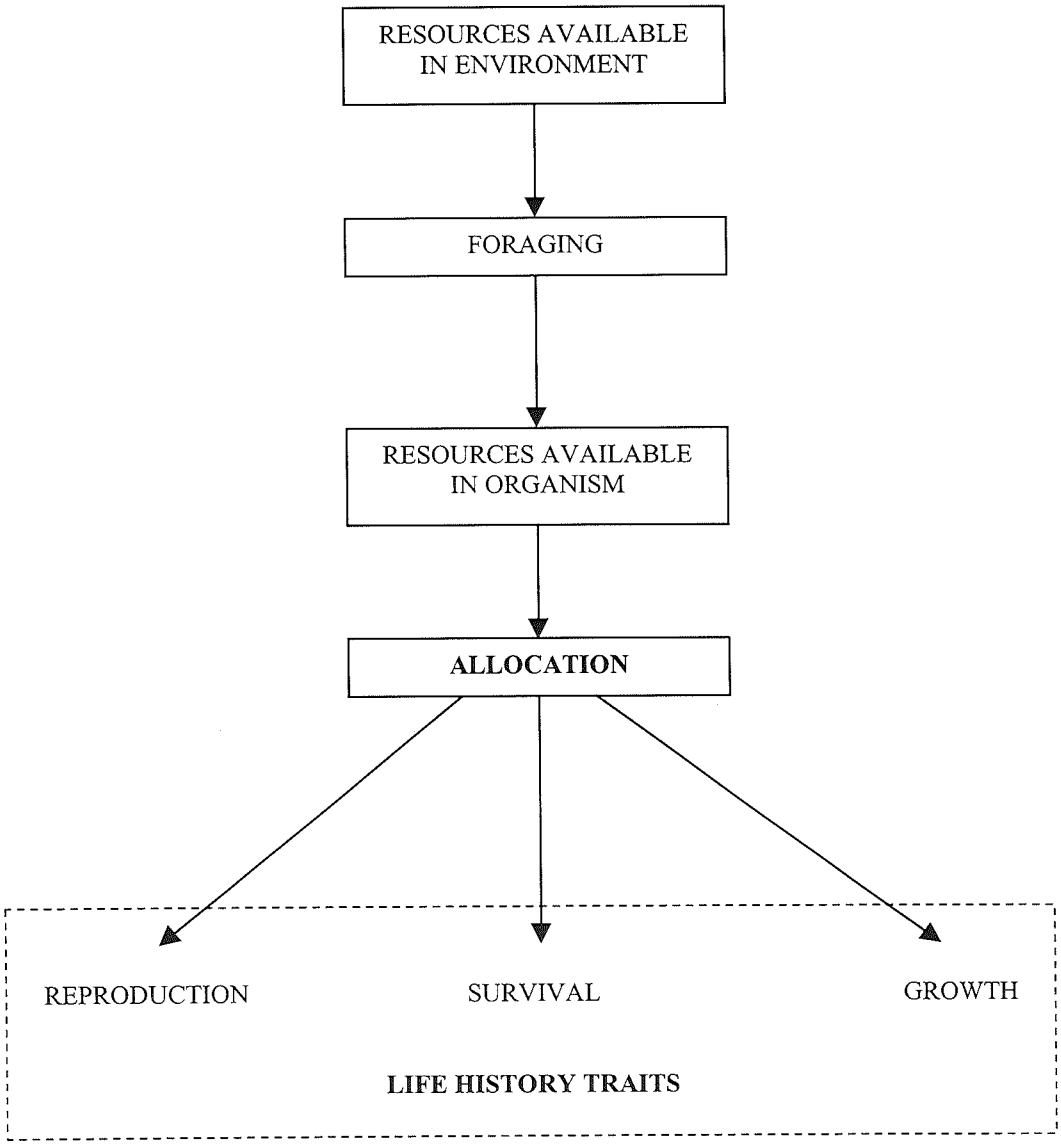

Fig. 1.1 The relationship between foraging, allocation and life history traits (reproduced from Boggs 1992).

manipulation of resource levels is required if trade-offs are to be uncovered (Partridge 1992, Stearns 1992, Roff 1992). However, as pointed out by Sinervo & Svennson (1998) manipulations of the environment (e.g. food availability) can also confound results because of genotype by environment interactions

Many trade-offs have been characterised (see Bell & Koufopanou 1986, Lessells 1991 and Stearns 1992 for reviews), and the ones that have received the most attention involve the costs of reproduction, i.e. that resources allocated to reproduction are not available to be allocated to growth, survival or future reproduction (Roff 1992, Stearns 1992). However, as well as the trade-offs that occur during the lifetime of individuals, competition for resources will also occur during the development of organisms.

1.1 Developmental trade-offs

In a similar way that reproduction can be classified into 'income' or 'capital' breeding strategies (Drent & Daan 1980), development can occur within an 'open' (e.g. the foetus of a placental mammal) or 'closed' system (e.g. an egg). In the former case, growing tissues and organs potentially have access to a continual supply of resources throughout development, either from a maternal source, or through feeding. Within 'closed' developmental systems, the resource pool available for growth is determined from the outset, and cannot be subsequently added to. As previously stated, it is because resources are generally limited that trade-offs occur. However variation in individual resource acquisition ability masks this and can lead to positive rather than negative associations between traits. Closed developmental systems therefore provide excellent models for measuring trade-offs as the limited nutrient pool is set from the outset and potential trade-offs cannot be subsequently masked by acquisition differences between individuals.

1.1.1 Development within an 'open' system.

Developmental trade-offs under these conditions have proved especially difficult to demonstrate, and so examples of such allocation trade-offs are rare. One of the few examples from vertebrates is in nestling barn swallows, where infestation

with ecto-parasites caused an increase in feather growth, which appeared to be traded-off against growth of both structural size and body mass (Saino *et al.* 1998). In the brittle star, *Ophiocoma echinata*, arm regeneration caused a decrease in lipid storage and smaller gonads (Pomory & Lawrence 1999). The following two examples demonstrate how manipulating resource levels can be used to demonstrate allocation trade-offs. When the essential nutrient choline was limiting in the diet of the spider, *Nephila clavipes*, a negative correlation was found between the amount in the body (cephalothorax) and the amount invested in the orb web (Higgins & Rankin 1999). Under-fed juvenile cockroaches, *Blattella germanica*, invest normal amounts of hydrocarbons in their adult exoskelteon but less internally (Young *et al.* 1999). This is also an example of how competition for a resource can produce trade-offs across more than one stage of the life-cycle.

One well-studied situation where trade-offs occur during development is the dispersal polymorphisms of hemimetabolous insects. These are insects where metamorphosis is incomplete, such that the transition from juvenile to adult occurs over a number of stages, rather than during the one, inactive pupal stage . It has been known for many years that in the different dispersal morphs of such insect species, flight ability is negatively associated with fecundity for females (Johnson 1969). Flightless morphs can have reduced wings (brachypters) or no wings (apters) and/or reduced flight muscle and reduced flight fuels, depending on the type of polymorphism (Zera & Denno 1997). As such, these morphs possess traits such as higher fecundity, earlier reproduction, lower mortality and larger eggs compared to flight capable morphs (macropters or alates) (Zera & Denno 1997). There is also some evidence for increased male reproductive success in flightless morphs (Langellotto *et al.* 2000 and references therein). The most widely held explanation for this is that the resources allocated to development and maintenance of the flight apparatus in the flight capable morph are reallocated to reproductive development in the flightless morph (Zera & Denno 1997).

Despite it usefulness as a system for investigating physiological trade-offs, development of adult hemimetabolous insects has two major draw backs when it comes to specifically looking at developmental trade-offs in that, (1) feeding and development are concurrent, and (2) muscle and ovarian growth can occur at different stages of the ontogeny. In certain situations where growth and development

occur under conditions of nutrient input, developmental trade-offs will be hard to detect, and indeed they may only occur under nutrient stress. For dispersal polymorphic species, different morphs sometimes have different consumption rates, which makes developmental trade-offs hard to detect (Mole & Zera 1994). Also, flight muscle development and ovarian growth are temporally separated in some species; there may be no muscle growth in the adult stage, which would preclude a direct developmental trade-off (although, there could be a trade-off through storage proteins from the juvenile to adult stage). The trade-off with reproductive effort observed in such species appears to be related more to the metabolic costs of maintaining a flight apparatus than to its construction (Zera & Denno 1997). Growth dynamics during ontogeny could therefore potentially confound the interpretation of developmental trade-offs.

1.1.2 Development within a 'closed' system.

When development occurs within a closed system however, the developing body parts are all formed from the same pool of resources (Trumbo 1999). So it is expected on theoretical grounds that competition will arise between them (Nijhout & Wheeler 1996), especially between organ systems developing at the same time. The existence of such trade-offs has, however, been difficult to demonstrate empirically. The main systems where development occurs inside a sealed environment are within eggs after oviposition, within the pupae of holometabolous insects, and within the non-feeding stages of certain other metamorphic organisms (e.g. amphibians, lecithotrophic marine invertebrates). As previously stated, one way to clearly demonstrate trade-offs is by direct manipulations of the phenotype. Experimentally manipulating egg composition after laying in birds and reptiles (by removing albumen and/or yolk) has generally resulted in structurally smaller hatchlings (Hill 1993, Sinervo 1993), but any allometric affects on overall embryo morphology have hardly ever been considered. In one study on chickens, although tibiotarsus length was shorter in the manipulated individuals, the mass of the gizzard and liver was unaffected (Finkler *et al.* 1998). This may imply a resource allocation trade-off between investment in the skeleton and investment in organ formation for the limited resources within the egg. Similarly, egg manipulations in marine invertebrates have

resulted in changes to body parts (L. McEdward *pers.com.*), but so far there has been no clear demonstration of trade-offs in investment.

1.2 Holometabolous insects

Most of the work on developmental trade-offs has in fact been carried out on holometabolous insects. Holometabolous insects undergo complete metamorphosis such that the life cycle consists of an egg, larva, pupa and adult. The adult form is constructed during the non-feeding pupal stage from breakdown of the larval body. During metamorphosis many adult structures such as legs and wings grow from differentiated areas of cells that are set aside during embryogenesis (Stern & Emlen 1999), and become either patches of the larval epidermis or pockets of cells (imaginal discs) within the body cavity (Nijhout & Emlen 1998). These cells only start to proliferate during the latter stages of larval life, mainly after the cessation of feeding, i.e. within a closed system (Williams 1980). Although natural selection will have shaped the pattern of resource allocation that occurs during metamorphosis, limitation in nutrient levels will also have an effect on adult morphology as different structures will be in competition for the same resources (Nijhout & Emlen 1998).

The size of an organ relative to body size or relative to another organ during growth is termed Ontogenetic Allometry (Schlichting & Piggliucci 1998). Evidence from recent experimental studies suggests that growing organs interact to regulate their relative sizes, through the sharing of limited resources (Stern & Emlen 1999, Day & Lawrence 2000), and much of the competition between organs may be determined by developmental dynamics. Competition may arise between growing body parts because they are spatially or temporally constrained. Body parts developing in close proximity may utilise resources from the same discrete pools, or resources may be compartmentalised so that competition between growing organs occurs because they are growing at the same time or rate (Nijhout & Emlen 1998, Blouin & Brown 2000). Removal of the imaginal discs that give rise to the hindwings in the buckeye butterfly, *Precis coenia*, caused an increase in resource allocation to the forewings, forelegs and thorax, but not the abdomen or head of the adults (Nijhout & Emlen 1998). Removal of the wing discs from just one side of the body produced an enlargement of the same structures, but only on that side

(Klingenberg & Nijhout 1998). This also implicates this mechanism as being involved in the asymmetry recorded in many of the paired bilateral traits of organisms (Møller & Swaddle 1997). Work on beetle species that are dimorphic for male horn size, has found that individuals which develop large horns have either smaller eyes (Emlen 1996, Nijhout & Emlen 1998) or wings (Kawano 1995), which implies that development of horns somehow restricts the expression of other morphological traits. Interestingly diet also affects horn allometry. In the beetle *Onthophagus acuminatus* a poor quality larval diet leads to smaller males with proportionally larger horns (Emlen 1997). Evidence from genetic manipulation also suggests the existence of developmental trade-offs. Mutations in *Drosophila* have produced similar results to phenotypic manipualtions. Individuals with large wings and halteres have reduced eyes, whilst those with enlarged eyes have a reduced scutellar size (see Hodin 2000 for references).

Apart from developmental constraints, life history will also be important in shaping development and resource allocation decisions (Grbic & Strand 1998). Gage (1995) raised larvae of the moth, *Plodia interpunctella*, in a range of densities. At low densities, males emerged with greater allocation to dispersal and mate finding ability (i.e. larger flight muscles), whilst at high densities they invested more in testes size, due to the (perceived) higher levels of sperm competition. The different seasonal forms of some butterfly species also invest differently in soma depending on their requirements for a long or short life span (Karlsson & Wickman 1989, Bradby & Jones 1995).

1.2.1 Resource allocation and life history.

Developmental trade-offs will have therefore have important consequences for the evolution of morphology, resource allocation and life history. The allocation of resources within holometbaolous insects to either maintenance or reproduction, and their relationship to life history has been well studied in butterflies. During metamorphosis, larval reserves are redistributed between reproductive and somatic tissues, within a closed system, and so there is potentially an allocation trade-off between them. How this trade-off is resolved will have profound affects on life history. Adult feeding is quite often minimal in such species, so abdominal size at

eclosion is often used as a predictor of reproductive potential, and somatic investment can be considered to be those resources allocated to the rest of the body, which is mainly the thorax (Boggs 1981). This makes many butterflies ideal for investigating allocation decisions, because they are expressed very obviously in the general adult morphology (Boggs 1981, Karlsson 1995, Rutowski 1997).

For instance, investment in thoracic tissue (which is mainly flight muscle) is high in species or individuals with high levels of expected flight activity (Marden & Chai 1991). Situations that require improved flight ability are predator avoidance (e.g. Srygley & Kingsolver 2000 and references therein) and mate location (e.g. Wickman 1992). Long lived adults, particularly those reproducing after undergoing a summer or winter diapause, also allocate proportionately more of their resources to the thorax (i.e. somatic investment) at the expense of reproductive investment, than those with a relatively short adult life-span (Karlsson & Wickman 1989, Bradby & Jones 1995). Mating system primarily influences the allocation of resources to the abdomen. Female butterflies range from monandrous to polyandrous (Svärd & Wicklund 1989, Gage 1994, Karlsson 1995) depending on the species, and the degree of polyandry also affects male mating frequency (Bissoondath & Wiklund 1996). Males of polyandrous species are known to invest heavily in reproduction under the threat of sperm competition, in terms of both sperm numbers (e.g. Parker 1982, 1998; Gage 1994) and in nutrients ('nuptial gifts') supplied to the female (Vahed 1998). Females of such species have relatively smaller abdomens (because of the expectation of high male investments) when compared to females from monandrous species, where male reproductive expenditure is low (Svärd & Wicklund 1989, Gage 1994, Karlsson 1995).

1.3 Caddis flies (Trichoptera)

The relationships between life history and resource allocation are fairly well established in butterflies, and there has also been some work on moths (Rydell & Lancaster 2000, Morrow & Gage 2000). However, whilst there is a lot of information on such relationships within the Lepidoptera, there is no equivalent knowledge for the Trichoptera (Hoffman 1999, Arnqvist 2000). The Lepidoptera and Trichoptera are sister taxa (Morse 1997), although the exact relationship is still unresolved. There

is somewhere in the region of 10,000 extant species of Trichoptera world-wide, divided into 600 genera within 58 families (Morse 1997).There are nearly 200 species found in Great Britain (Wallace *et al* 1990). Almost all species have aquatic eggs, larvae and pupae, and terrestrial adults. The larvae inhabit a wide range of lentic and lotic habitats (Mackay & Wiggins 1979) where they usually make up a significant proportion of the benthos.

Like Lepidopteran larvae, caddis fly larvae also have the ability to produce silk from modified labial glands. This has allowed the diversification in feeding habits, defence and habitat selection that caddis fly larvae display. Silk utilisation differs between families, and can be broadly divided into three modes (Mackay & Wiggins 1979). Families such as the predatory Rhyacophiloidea, spin only a thin thread whilst moving over the substrate, presumably to prevent accidental entry into the drift. More sedentary larvae, such as the Hydropyschoids, spin nets or shelters which are used in food capture. The most significant use of silk is in the construction of mobile cases, by families such as the Limnephillidea. Cases are constructed from organic or mineral material found in the immediate environment of the larvae. Case building is usually species, and sometimes instar specific, in terms of size, shape and material. The variation in case building seen across the Trichoptera has led to the proposal of a number of explanations as to the function of the larval case . These include aiding respiration (Wiggins 1977), resistance to high current velocities (Dodds & Hishaw 1925, Hynes 1970 Delgado & Carbonell 1997), protection from desiccation (Zamora-Munoz & Svensson 1996) and defence against parasites (Gallepp 1974). However, it is defence against predators that is the most likely function (e.g. Otto & Svensson 1980, Johansson 1991, Johansson & Johansson 1992, Nislow & Molles 1993).

Despite their obvious differences (mainly the long, aquatic larval stage in caddis flies compared to the shorter, terrestrial larval stage in butterflies and moths) Trichopterans and Lepidopterans have a very similar biology, particularly with regard to the adult stage. As with Lepidopterans, caddis flies undergo a complete reorganisation of the body during metamorphosis, and emerge into a flying adult form with a nutrient limited diet (e.g. Baker & Baker 1973, 1986; Petersson & Hasselrot 1994). The males of many species also produce spermatophores (Khalifa 1949, Malicky 1973, Petersson 1991) as do butterfly species. However, most work

on reproductive behaviours has centred on swarming behaviours in Leptocerids (e.g. Solem 1978, Petersson & Solem 1987). Some authors have reported multiply mated females in the wild, for example Svensson (1972) found females of Sericostomatidae and Limnephilidae with more than one sperm sac, but actual measures of degree of mating frequency, parental investment and general mating strategy are unknown for most families (Hoffmann 1999).

1.4 Aims

This thesis consists of an investigation into whether developmental trade-offs in resource allocation occur during the metamorphosis of holometabolous insects, and specifically whether trade-offs exist between somatic and reproductive investment. The extent to which resource allocation decisions can be altered by environmental conditions prior to pupation, or by life style requirements subsequent to body construction at pupation, are also examined. Finally, by using the relationship between body size and shape with life history known from the closely related butterflies, an attempt is made to predict aspects of the life history of adult caddis flies from measurements of resource allocation.

Chapter 2 aims to determine the existence of developmental trade-offs during the pupation of the case building caddis fly *Odontocerum albicorne*. One potential way to uncover developmental trade-offs is to manipulate resource levels before the formation of tissues and organs begins, and observe the affect on the resultant body form. This is attempted by increasing the demands of case building by removal of the larval case from individuals in their final instar, just prior to pupation. For caddis fly larvae the main cost involved in case construction is silk production. Silk production is a considerable drain on resources, in terms of both energy (Otto 1974) and specific compounds (Berenbaum *et al.* 1993, Craig *et al.* 1999, Higgins & Rankin 1999), and its production is likely to have negative effects for growth and development (Dudgeon 1987, Jakob 1991). Not only that, but removing larval cases also allows investigation of the trade-off between larval silk production and resources for the adult form, as larvae will normally be faced with the conflict of allocating finite resources into protection from predators or channelling them into growth.

Having established in Chapter 2 the existence of developmental trade-offs within a species with short lived adults, an investigation is then made to determine whether the observed affects on the adult body are a consequence of fixed developmental pathways, or whether there is some flexibility in allocation patterns (**Chapter 3**). Evidence from studies on the butterfly *Precis coenia* suggest that the resources liberated from the breakdown of larval structures during metamorphosis, enter into discrete 'pools' that are only available to certain adult structures developing spatially and/or temporally near (Nijhout & Emlen 1998). If this were the case then the results of the resource depletion experiment from Chapter 2, repeated on a related species (*Glyphotaelius pellucidus*), would be expected to be similar. However, if the allocation of resources were in some way strategic, such that individuals can minimise the effects of resource depletion on adult fitness, then the pattern would be expected to be different, as the two species examined differ quite markedly in life history strategy, particularly adult life span.

Although investment in somatic and reproductive tissues is generally determined at pupation, the consequences of particular resource allocation strategies and the way in which body resources are utilised post-emergence, is poorly understood even within butterflies (Stjernholm & Karlsson 2000). Adult caddis flies have proved difficult to study, mainly due to their nocturnal behaviour (Halat & Resh 1997). One way of assembling reasonably large quantities of adults is to operate a light trap (Crichton 1971, Svensson 1972). In **Chapter 4** the results of a three-year study on the species collected in a light trap, based at the University Field Station, are presented. The numbers and flight periods for both sexes, as well as a description of their larval ecologies are provided. Some of the species collected in the first year of that study, as well as some other individuals reared in captivity are then used for an analysis of resource dynamics (**Chapter 5**). Recent evidence from butterflies suggests that in many species, the flight muscles are broken down to provide extra nitrogen for reproduction, which is a limiting resource due to its absence from the adult diet, but its high content in spermatophores and eggs. This pattern in butterflies has been found to be dependent on both sex and the type of mating system (Karlsson 1994, 1998; Stjernholm & Karlsson 2000).

As well as the potential changes in resource allocation patterns during adult life, environmental conditions encountered during the larval stage could also

potentially affect resource allocation patterns. It is well known that seasonal changes in temperature and food quality, cause a decrease in body size at maturation over time for many univoltine arthropods (Sebens 1987). However, to what extent changes in structural body size are mirrored by changes in resource allocation patterns is unknown. In **Chapter 6** body size, and resource allocation patterns are measured in two caddis fly species with long emergence periods. In species such as these, adults that emerge early in the year will have experienced different conditions to those emerging later.

Patterns of resource allocation within adult butterflies have been well studied with respect to their relationship with certain aspects of the life history. These include such features as flight ability, longevity and mating system (e.g. Srygley & Kingsolver 1998, Karlsson & Wickman 1989, Karlsson 1995). The life history of adult caddis flies is however poorly known (Halat & Resh 1997, Hoffmann 1999, Arnqvist 2000). In **Chapter 7** theories developed for butterflies are used to make predictions about the life history of adult caddis flies based on resource allocation patterns. **Chapter 8** is a synthesis of all the chapters and contains suggestions as to how developmental trade-offs and life history strategies might operate to shape resource allocation patterns in holometabolous insects such as butterflies and caddis flies.

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Chapter 2. Developmental trade-offs in caddis flies: increased investment in larval defence alters adult resource allocation.

This chapter is an expanded version of Stevens, D.J., M.H. Hansell, J.A. Freel & P.Monaghan 1999 Developmental trade-offs in caddis flies: increased investment in larval defence alters adult resource allocation. *Proc. R. Soc. Lond.* B **266**, 1049-1054.

2.1. INTRODUCTION

The concept of trade-offs between traits is a central component of life history theory. Given that the resources available to individuals are generally finite, increased allocation of resources to one trait will be at the expense of those allocated to other competing traits (Stearns 1992). In addition to the trade-offs that occur during the lifetime of the individual, for example between activities related to survival and reproduction, trade-offs may occur during development in the allocation of resources between different body parts, within the individual itself. The existence of such developmental trade-offs, and the ways in which they are constrained by developmental mechanisms, have important consequences for the evolution of morphology (Trumbo 1999). Furthermore, in species that go through a number of life history stages, increased expenditure of finite resources during one life history stage may have profound consequences for the pattern of allocation of resources to different body parts during subsequent stages.

There are a number of different approaches to the study of life-history trade-offs, and experimental manipulations of the phenotype can give a valuable insight into the nature of particular trade-offs and their functional significance (Partridge 1992, Roff 1992, Stearns 1992). While the concept that different parts of the organism may be in competition with each other for resources during development is well established, this has proved very difficult to demonstrate experimentally (Nijhout & Emlen 1998, Trumbo 1999). Hormonal manipulations that influence the growth trajectories of particular organs or organ systems, thereby altering the resources available to other developing body parts, have recently proved a very useful tool in studying developmental trade-offs and the underlying endocrine regulatory mechanisms (Ketterson and Nolan 1992, Sinervo 1993, Zera et al. 1998). The complexity of endocrine interactions, and the existence of compensatory feedback loops, however, can make the interpretation of the results difficult (Zera et al. 1998).

A complementary approach is to alter the resources available to organisms prior to organ formation and examine the effect on subsequent development (Sinervo 1993). Holometabolous insects, where organ development takes place in a closed system (the pupa), provide an excellent opportunity to study developmental trade-offs

if the resources available to the larva at pupation can be manipulated. Furthermore, altering the demands of particular activities during the larval stage, and examining the effects on the adult, gives the additional opportunity to examine trade-offs in the allocation of resources between life history stages.

Caddis flies are holometabolous insects in which there is a distinct larval stage in fresh water, followed by pupation and the emergence of a free-flying, largely non-feeding, adult reproductive form (Hickin 1967). Many species of caddis fly build portable cases of organic and/or inorganic material, held together with silk secreted by the larva. The main function of these cases is protection from predators (Otto & Svensson 1980), usually fishes and aquatic invertebrates. Such case building requires a relatively high investment by the larva in silk production. Otto (1974) estimated that this amounted to about 12% of the total larval energy content in the case building caddis *Potomophylax cingulatus*, and silk synthesis costs have also been found to be significant in various other silk-producing taxa (Prestwich 1977, Dudgeon 1987, Jakob 1991, Berenbaum *et al* 1993).

In insects such as caddis flies, where adult feeding is minimal, the formation of the adult and its subsequent condition will depend on the amount of stored reserves acquired by the larva. Therefore, resources that are diverted into larval defence, such as case building, will not be available for utilisation by the adult. Nitrogen is an important resource in this respect. It is a major constituent of silk (Craig 1997), it is absent from the adult diet even if some feeding does occur, and muscle, eggs (vitellogenin) and spermatophores (lipo-protein) are all high in nitrogen (Boggs 1981). In holometabolous insects, allocation to either adult soma or reproduction occurs during pupation and is fixed. The amount of resources available for reproduction is approximately equivalent to the amount of resources allocated to the abdomen at eclosion (Boggs 1981), whilst the amount allocated to soma is equivalent to that allocated to the rest of the body, which is mainly the thoracic flight muscles (Karlsson & Wickman 1989).

To determine the effect of increased demands at the larval stage on the pattern of resource allocation in the developing adult, we experimentally manipulated the demands of case building in the mineral case building caddis fly *Odontocerum albicorne*. Since it is important to establish the extent to which the organism responds

by any changes in resource acquisition, as well as resource allocation, we monitored the food intake of the larvae from the manipulation until pupation. Resources allocated to defence were measured as the amount of silk contained within larval cases. The allocation of resources to the somatic and reproductive body components of the adults was examined in recently emerged individuals, in terms of both mass and nitrogen.

2.2. METHODS

Larval *Odontocerum albicorne* are found in rivers with gravel and sand substrates, and are omnivorous scavengers (Elliott 1982). The case is composed of sand grains, and silk is applied as 'mortar' and braces between grains (Wallace *et al.* 1990), giving a very strong end product (Otto & Svennsson 1980). The larval growing period is relatively long. Eggs hatch in the autumn and adults emerge in late summer and live for less than two weeks.

Seventy-seven fifth-instar *O.albicorne* larvae were collected on the 18th May & 2nd June 1997 from a small burn in Milngavie, Glasgow and transported to the University Field Station, Rowardennan, Loch Lomond. Individual larvae were removed from their cases, blotted dry, weighed and randomly assigned to either the control or experimental group. There was no difference in the weights of larvae allocated to each group at the start of the experiment; however, as is typical of this species (Elliot 1982), male larvae were significantly smaller than females (Fig. 2.1.). Controls were placed back into their original cases, whereas experimentals were not, thus obliging them to rebuild. A few larvae in both the control and experimental groups died shortly after collection, escaped or did not complete development; these were excluded from further analysis and thus sample sizes vary accordingly. The proportion lost did not differ between control and experimental groups. Animals were housed singly in small plastic pots (diameter 82 mm x height 57 mm) with gauze lids, which were placed in a small outdoor flume (270 cm x 126 cm x 32 cm) (Fig. 2.2.). Sand from the collection site was added to each pot for use as building material and as a substrate to facilitate easy movement. Once all experimental animals had rebuilt a new case (three days), four frozen blood worms were provided as food for individuals of both groups. Every few days all the pots were checked to record how much food had been eaten. Uneaten food items were removed, and fresh worms added.

Larvae were considered to be in pupation once they had closed off the opening of their case with a small stone. Once larvae entered pupation, they were removed from the flume and placed individually into the numbered cells of plastic trays in another tank that

had water flowing through it, and were covered by a net to catch newly eclosed adults. Adults emerged overnight and were collected from the net sometime the next day. They were then frozen, and the remaining empty pupal cases were retained for further analysis.

Frozen adults were weighed and right fore-wing length was measured using a microscope with an eyepiece micrometer. Heads, legs and wings were removed and the remaining thorax-abdomens were dried to a constant weight at 60^0 C, separated and then weighed. Nitrogen content, which is a good measure of protein content (Gnaiger & Bitterlich 1984), was measured by flash combustion of dried thoraces and abdomens in a Carlo Erba 1106 elemental analyser. All larval and pupal cases were freeze-dried, weighed and combusted using a bomb calorimeter. The remaining sand grains were then weighed, and the silk content of the case calculated by subtraction. To comply with the requirements of the statistical tests used, all data were log transformed.

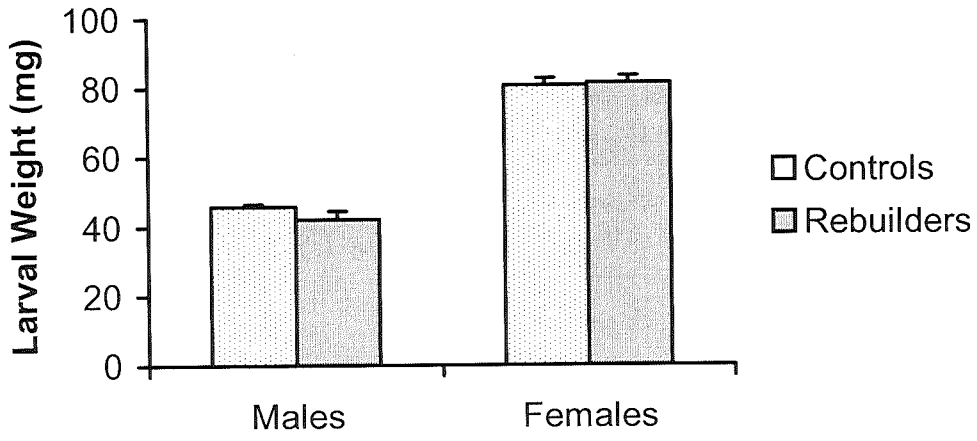

Fig. 2.1 Mean larval weights (with SE bars) at the start of the experiment. Weights did not differ between the groups, although females were larger than males (GLM: Sex $F_{(1,43)}$ = 186.71, P<0.001; Group $F_{(1,43)}$ = 1.25, $p > 0.05$).

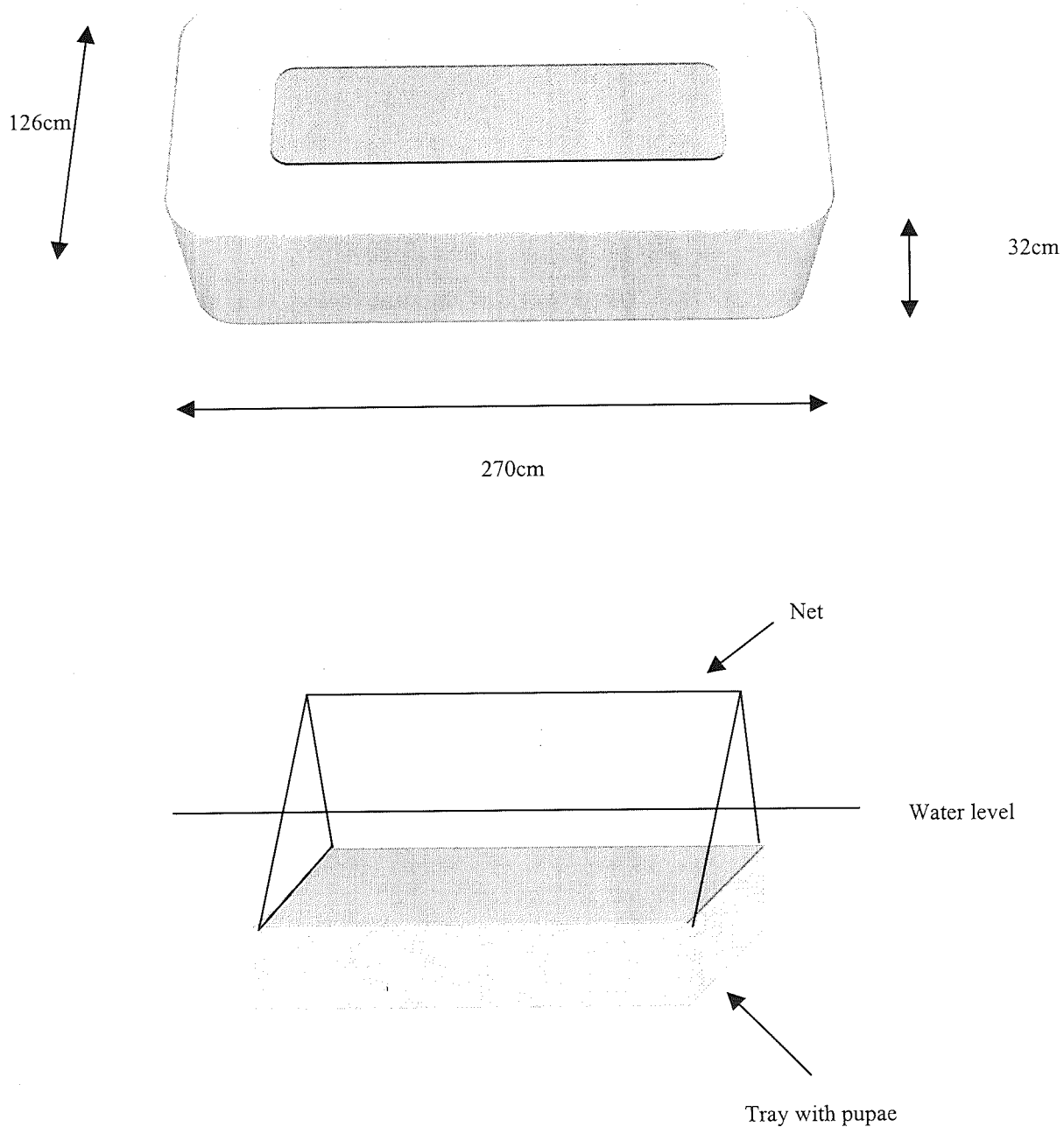

Figure 2.2 (a) The recirculating flume that housed larvae and (b) a net-covered tray use to capture emerging adults.

2.3. RESULTS

2.3.1. Larval Cases

By the end of the experimental period, cases of the control group were significantly heavier than the original cases removed from rebuilding larvae at the start of the experiment (Fig. 2.3). This reflects the further building the larvae undertook as they grew, between the time of the start of the experiment and the time of entering pupation. Silk comprised a similar proportion of these cases (Fig. 2.4). Control cases and rebuilt cases did not differ in mass at the point of adult emergence, which indicates larvae rebuild cases to a size consistent with their own body size (Fig. 2.3). The percentage silk content was lower, however, in the rebuilt cases in comparison (Fig. 2.4). Total silk production by individuals in each group was taken as the silk content of the final case (controls) and the silk content of the rebuilt case plus that of the removed case (rebuilders). Total silk expended by larvae was significantly different between the treatment groups for both males and females, demonstrating the extent of the increased investment in silk required by the experimental group (increased on average by 53% in males and 60% in females, Fig. 2.5).

2.3.2. Food intake & development rate

There was no change in resource acquisition by rebuilding larvae, as the mean amount of food eaten per day (Fig. 2.6) did not differ between the treatment groups or sexes. Amount eaten was however highly dependent on the larval weight at the start of the experiment, with the smallest individuals of each sex consuming the most (Fig. 2.7). There was also no difference between the groups or sexes in the mean date of pupation (Fig. 2.8). The time spent in the pupal stage differed between males and females, and was reduced in both to a similar extent in the experimental group (Fig 2.9).

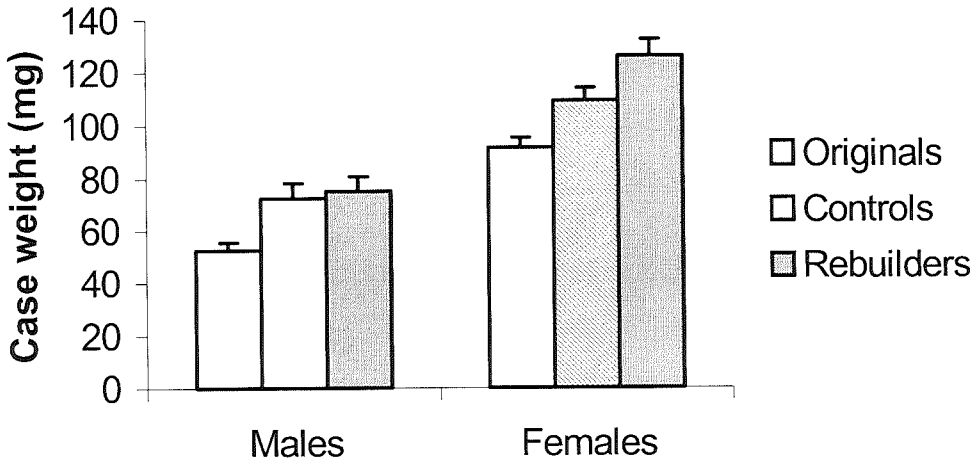

Fig. 2.3 Case weights of larvae (means with SE bars). The case weights of controls at pupation were significantly greater than those removed from the experimentals at the start of the experiment ('originals') (GLM: Sex $F_{(1,60)} = 72.09, p < 0.001$; Group $F_{(1,60)} = 17.88, p < 0.001$; Interaction $F_{(1,60)} = 0.05, p > 0.05$). However, case weight at pupation did not differ between controls or rebuilders. (GLM: Sex $F_{(1,42)} = 58.05, p < 0.001$; Group $F_{(1,42)} = 3.00, p > 0.05$; Interaction $F_{(1,42)} = 1.47, p > 0.05$)

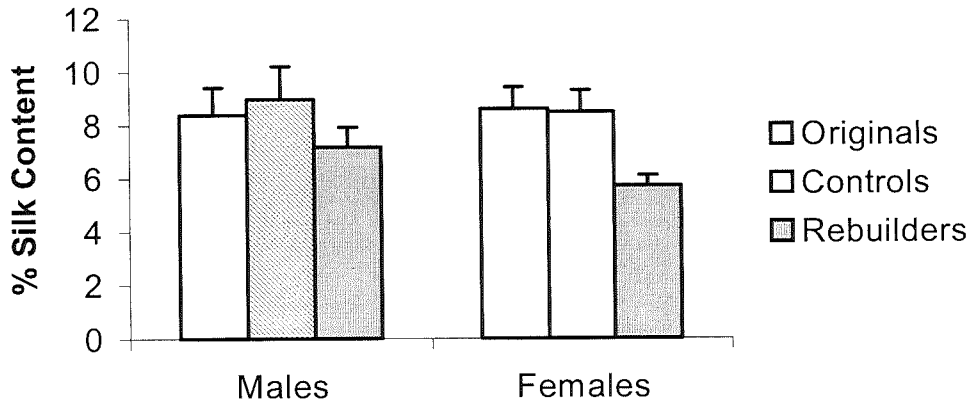

Fig. 2.4 Mean case silk content (with SE bars). Cases removed at the start of the experiment and control pupal cases did not differ in silk content (GLM on silk mass with case mass as covariate: Covariate $F_{(1,58)} = 10.73, p < 0.01$ Sex $F_{(1,58)} = 1.02, p > 0.05$; Group $F_{(1,58)} = 0.94, p > 0.05$; Interaction $F_{(1,58)} = 0.56, p > 0.05$). However, the pupal cases of rebuilders contained a significantly smaller proportion of silk (GLM on silk mass with case mass as covariate: Covariate $F_{(1,45)} = 7.94, p < 0.01$ Sex $F_{(1,45)} = 0.00, p > 0.05$; Group $F_{(1,45)} = 6.01, p < 0.05$; Interaction $F_{(1,58)} = 0.61, p > 0.05$)

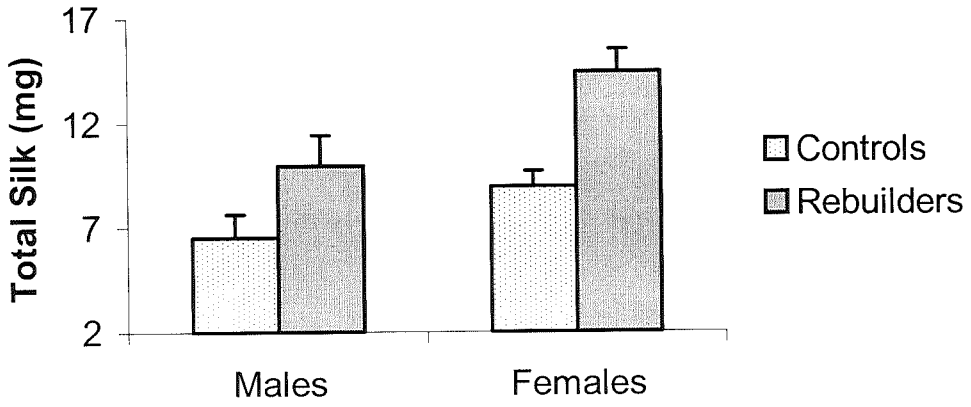

Fig. 2.5 Mean total silk production (with SE bars). Silk production was much greater for the rebuilding group (GLM: Sex $F_{(1,40)} = 13.11, p < 0.001$; Group $F_{(1,40)} = 20.00, p < 0.001$; Interaction $F_{(1,40)} = 1.00, p > 0.05$)

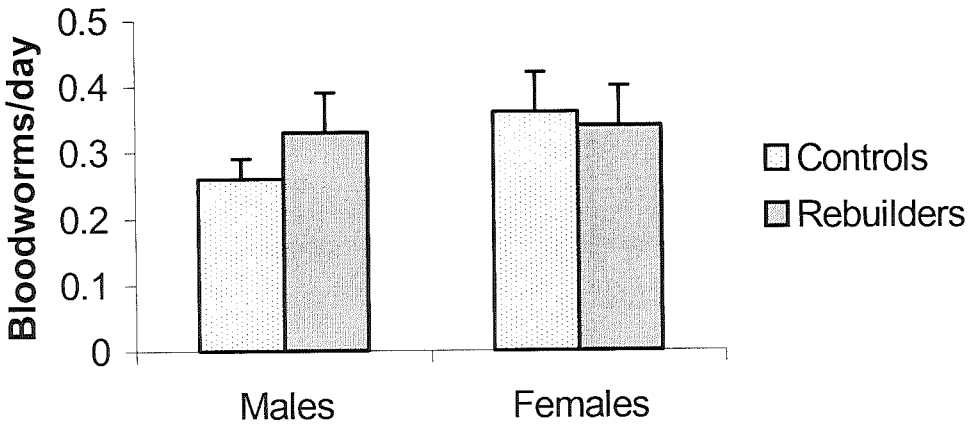

Fig. 2.6 Mean daily food intake (with SE bars). Food intake was not affected by sex or group (GLM: Sex $F_{(1,57)} = 0.10, p > 0.05$; Group $F_{(1,57)} = 0.21, p > 0.05$; Interaction $F_{(1,57)} = 0.31, p > 0.05$)

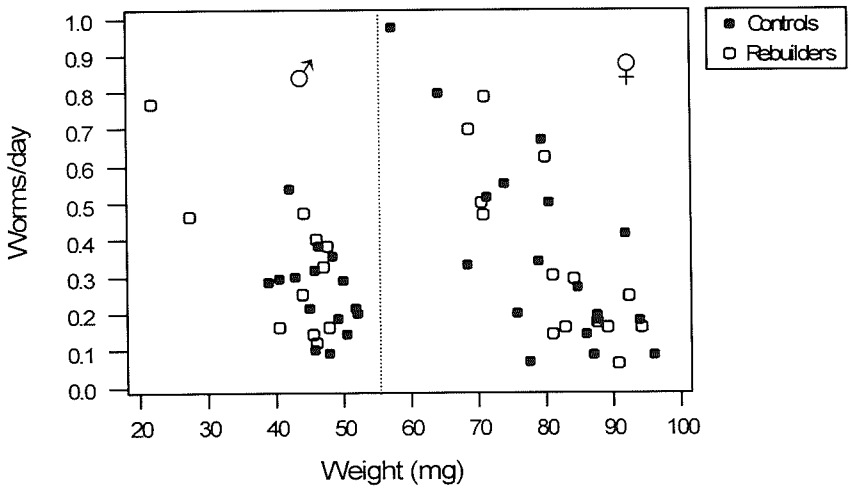

Fig. 2.7 The relationship between larval weight at the start of the experiment and the subsequent average daily food intake. There was a significant negative relationship within both the males (r = -0.659, n = 26, $p < 0.01$) and females (r = -0.764, n = 32, p <0.01)

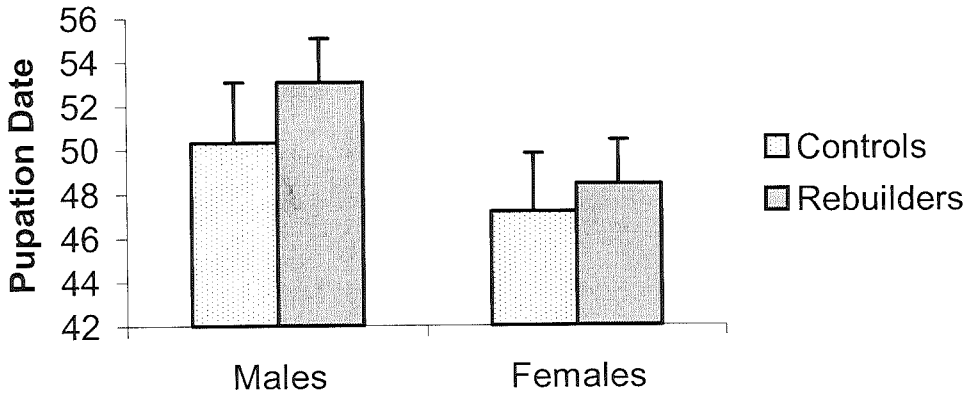

Fig. 2.8 Mean pupation date (with SE bars). The date of entering pupation (in days after the start of the experiment) did not differ between groups or sexes (GLM: Sex $F_{(1,57)} = 2.17$, $p > 0.05$; Group $F_{(1,57)} = 1.00$, $p > 0.05$; Interaction $F_{(1,57)} = 0.06$, $p > 0.05$).

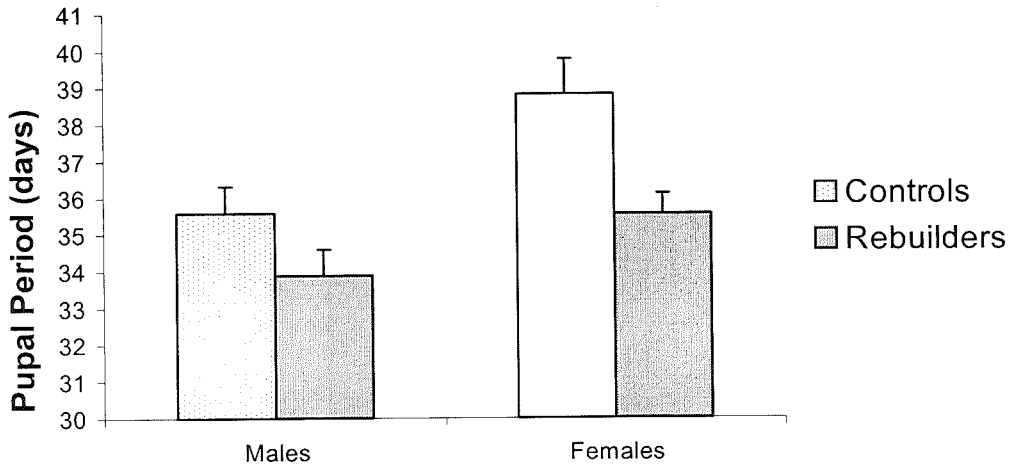

Fig. 2.9 Mean pupal duration (with SE bars). The pupal duration was shorter in males than females, and rebuilding the case shortened it for both sexes (GLM: Sex $F_{(1,57)} = 8.13$, $p < 0.01$; Group $F_{(1,57)} = 8.12$, $p < 0.01$; Interaction $F_{(1,57)} = 0.60$, $p > 0.05$).

2.3.3. Effects on adult resource allocation

The increased allocation of larval reserves to case building was associated with a change in adult morphology. In both control and rebuilding groups, females were larger than males (Fig. 2.10). However, fore-wings of adults in the rebuilding group were shorter in both sexes (Fig. 2.11) and their thoraces lighter on average than those of the controls (Fig. 2.12). The proportion of nitrogen in the thoracic tissue did not change (Fig. 2.13). Abdomens on the other hand did not differ in size between the groups (Fig. 2.14). As with the thorax, the proportional nitrogen content of the abdominal tissue did not change (Fig. 2.15).

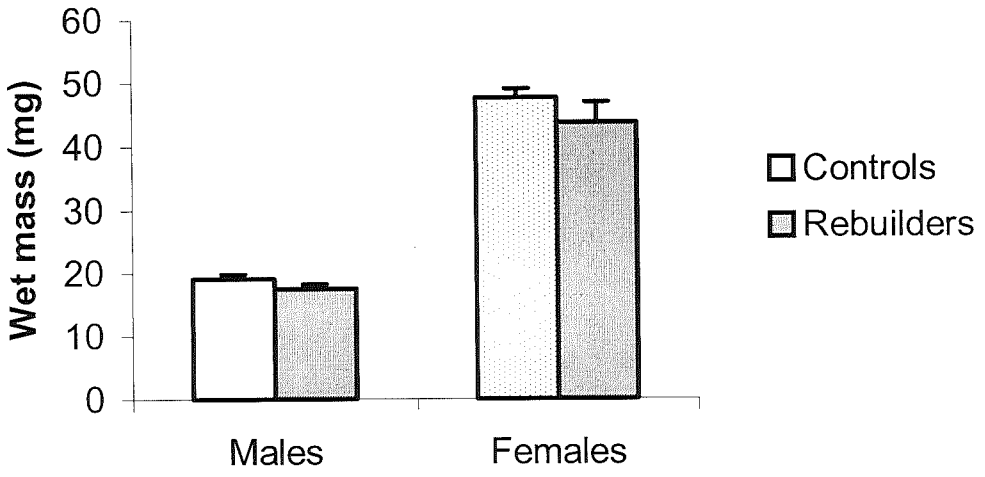

Fig. 2.10 Mean wet mass (with SE bars). The wet mass of freshly emerged adults was not significantly affected by rebuilding, but males were smaller than females (GLM: Sex $F_{(1,40)} = 235.63$, $p < 0.001$, Group $F_{(1,40)} = 2.48$, $p > 0.05$; Interaction $F_{(1,40)} = 0.48$, $p > 0.05$).

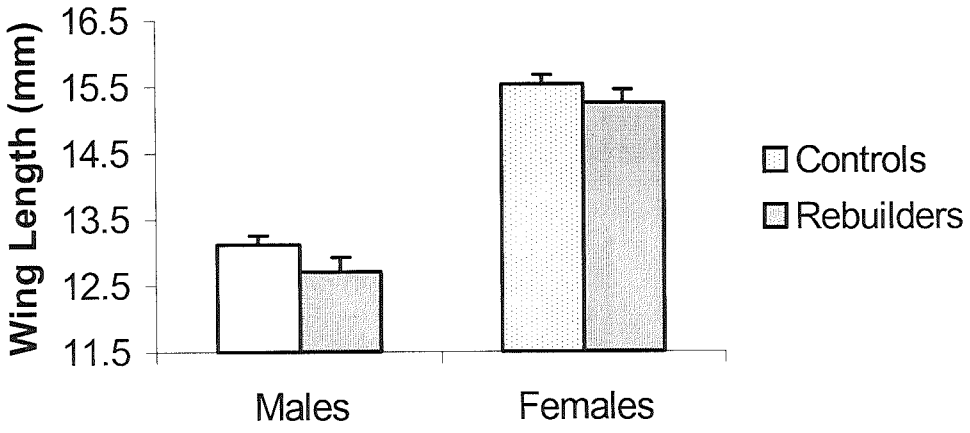

Fig. 2.11 Mean right fore-wing length (with SE bars). Males had shorter wings than females, and rebuilders of both sexes had shorter wings than controls (GLM: Sex $F_{(1,39)} = 215.87$, $p < 0.001$, Group $F_{(1,39)} = 4.39$, $p < 0.05$; Interaction $F_{(1,39)} = 0.30$, $p > 0.05$).

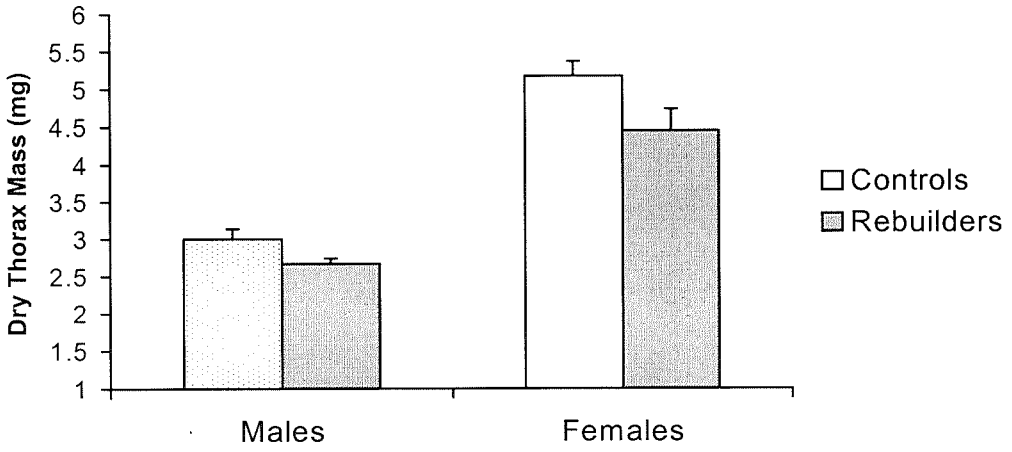

Fig. 2.12. Mean dry thorax mass (with SE bars). Males has lighter thoraces than females, and rebuilders thoraces were lighter than controls. (GLM: Sex $F_{(1,40)} = 105.64, p < 0.001$; Group $F_{(1,40)} = 7.33, p < 0.01$; Interaction $F_{(1,40)} = 0.27, p > 0.05$).

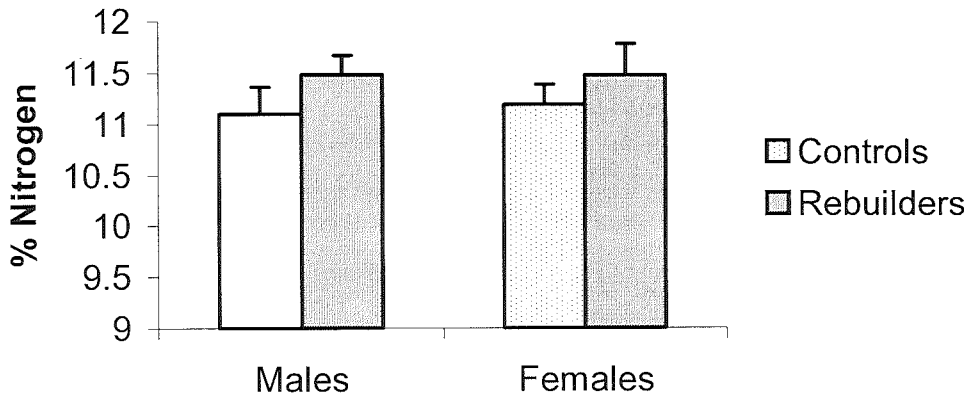

Fig. 2.13 Mean thoracic nitrogen content (with SE bars). Nitrogen content differed between the sexes, but did not differ between groups. (GLM on thoracic nitrogen content with thorax mass as covariate: Covariate $F_{(1,39)} = 146.27, p < 0.001$ Sex $F_{(1,39)} = 5.64, p < 0.05$; Group $F_{(1,39)} = 0.40, p > 0.05$; Interaction $F_{(1,39)} = 1.81, p > 0.05$)

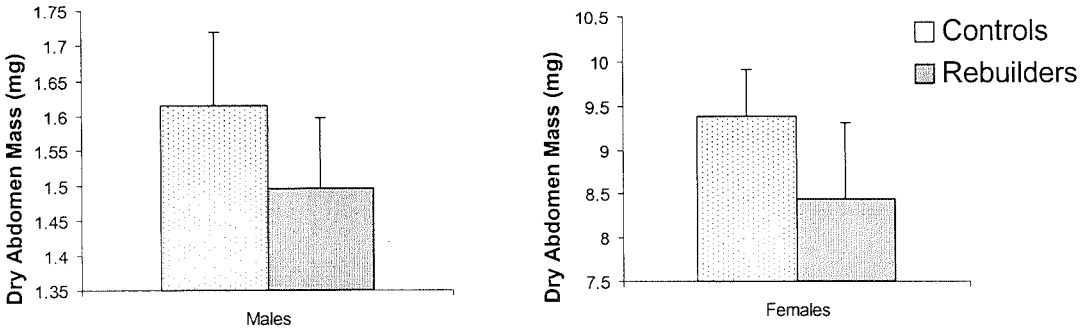

Fig. 2.14 Mean dry abdomen mass (with SE bars). Abdomen mass was much greater in females than males, but did not differ between sexes (GLM: Sex $F_{(1,40)} = 301.35, p < 0.001$; Group $F_{(1,40)} = 1.36, p > 0.05$; Interaction $F_{(1,40)} = 0.21, p > 0.05$)

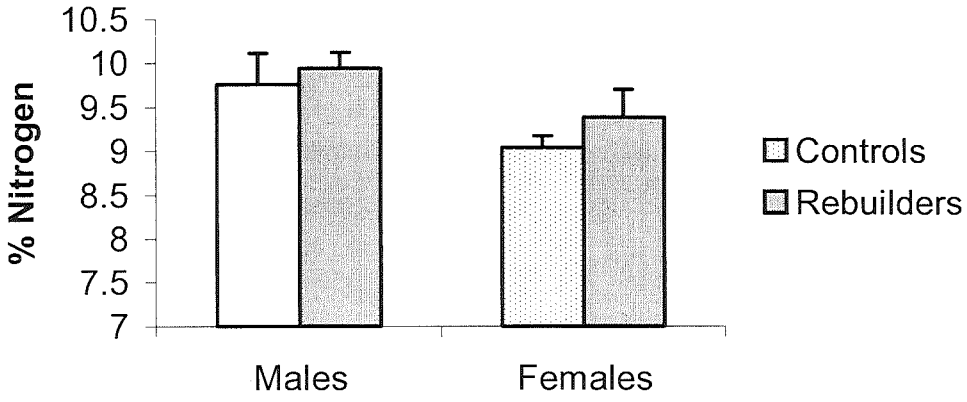

Fig. 2.15 Mean abdominal nitrogen content (with SE bars). Nitrogen content did not differ between sexes or groups. (GLM on abdominal nitrogen content with abdomen mass as covariate: Covariate $F_{(1,39)} = 333.19, p < 0.001$ Sex $F_{(1,39)} = 2.31, p > 0.05$; Group $F_{(1,39)} = 0.27, p > 0.05$; Interaction $F_{(1,39)} = 0.00, p > 0.05$)

2.4. DISCUSSION

The incorporation of silk into the larval case is vital for the defence of caddis fly larvae because silk allocation is positively correlated with larval security (Otto & Svensson 1980). Silk expenditure appears to be an important factor influencing life history strategies in caddis flies. It has been suggested, for example, that increased investment in silk is responsible for the relatively smaller larval size at pupation of caddis species making relatively expensive mineral cases, compared to those species making cheaper organic cases. (Otto 1982). The effect of intra-specific differences in larval silk expenditure has not hitherto been examined.

Rebuilt cases contained less silk at pupation than did the cases of control flies, indicating that case quality is reduced when cases are completely rebuilt in a matter of days, rather than over the course of the larval period. Despite this, overall silk expenditure was much greater in the experimental group. The consequent reduction in size of the thorax and wings in the adults could have various potential consequences. Flight is the most energy demanding activity performed by insects (Sartori *et al.* 1992). The thorax of holometabolous insects is mainly flight muscle (Petersson 1995), and a proportionally smaller thorax will sustain greater stresses during flight. Adult insects with a low flight-muscle ratio (thoracic mass/body mass) will have a reduced flight ability (Marden 1989), and distance flown and manoeuvrability have been shown to be greater in Lepidoptera species with high flight muscle ratios (Srygley & Kingsolver 1998). However, caddis flies are reluctant fliers and many species do not move far from the water body from which they emerged (Hickin 1967), so any effect of reduced thorax size and reduced muscle mass on flight performance may not be as important as other possible effects. Thoracic size has been found to be positively correlated with longevity in various Lepidopterans (Gage 1995, Karlsson & Wickman 1989). A reduction in longevity could have effects on realised fecundity, particularly in polygamous species. However, it may be that for caddis flies that do not have a long life-span and mate quite quickly after emergence (such as with *O. albicorne* used in this experiment), maintaining larval survival at the expense of adult life-span may be more important. Other species of caddis flies from temporary waters emerge as adults in spring or early summer (Hickin 1967) and undergo ovarian diapuase for several months before they are able to lay eggs (Novak & Sehnal 1963). Any decrease

in adult longevity would be expected to have much more important fitness consequences in these species, as they may suffer pre-reproductive mortality if adult life-span was reduced.

My finding that increased larval silk production shortened the duration of the pupal stage in the experimental group is probably because the time taken for tissue reorganisation is mass dependent. The resultant adult morphology, in which thorax size, but not abdomen size, was reduced could be a consequence of developmental constraints. Developmental pathways may be spatially constrained, such that the resources released from the breakdown of larval structures enter into discrete resource 'pools', each of which are designated for the building of specific body-parts only (Trumbo 1999). These resource pools may be composed of the breakdown products of anatomically close structures in the larva, and they may only give rise to body parts in similar areas to where they originated. In this study, silk glands will have been depleted after rebuilding the case; this may explain why the thoraces and wings were smaller, but not the abdomens in manipulated individuals, as silk glands also arise in the anterior part of the body. Alternatively, the constraint may be temporal, with resources liberated from the breakdown of larval structures only available to be incorporated into adult tissues that develop soon afterwards. Nijhout & Emlen (1998) found that removing the imaginal discs that give rise to the hindwings in *Precis coenia* larvae (Lepidoptera: Nymphalidae) caused an increase in resource allocation to the forewings, forelegs and thorax, but not the abdomen or head of the adults. This also would suggest the existence of compartmentalised pools of resources during pupation.

Conversely, the maintenance of abdomen size may represent a strategic preservation of reproductive potential in the face of diminished resources with which to form the developing adult. Allocation patterns have been found to be adjustable within holometabolous species depending on the predicted adult needs. Gage (1995) found that larval males of the moth *Plodia interpunctella* could alter allocation to abdomens or thoraxes depending on larval densities. At high larval densities - and therefore high adult densities - sperm competition is high and males allocate more to reproduction (testes size). At low densities, males allocate more to migratory and mate finding ability (i.e. the thorax), and consequently live for longer. Karlsson &

Wickman (1989) found that directly-developing individuals allocated less resources to their thoraces than hibernating (i.e. longer-lived) individuals in the Lepidopteran *Polygonia c-album*.

Differences between individuals in case-building requirements does occur in the wild, since case loss in larval caddis flies occurs under certain adverse conditions. For example, some species leave their cases as a result of strandings after spates (K. Hall & J. Lancaster *pers comm.*), after being accidentally buried in the substrate (Dobson *et al.* 2000) or during times of respiratory stress (Otto 1982) and new cases are built. The timing of case loss in relation to the timing of pupation may be crucial. My experiment was conducted in 5th instar larvae, relatively close to pupation, and thus the time available to compensate for the increase cost was relatively short. Increased investment in larval defence comes at the expense of adult thoracic size and protein content, which presumably means reduced muscle mass. Abdomen size however, is preserved. The results of this study provide an empirical demonstration of trade-offs that have long been speculated, but rarely demonstrated empirically (Trumbo 1999).

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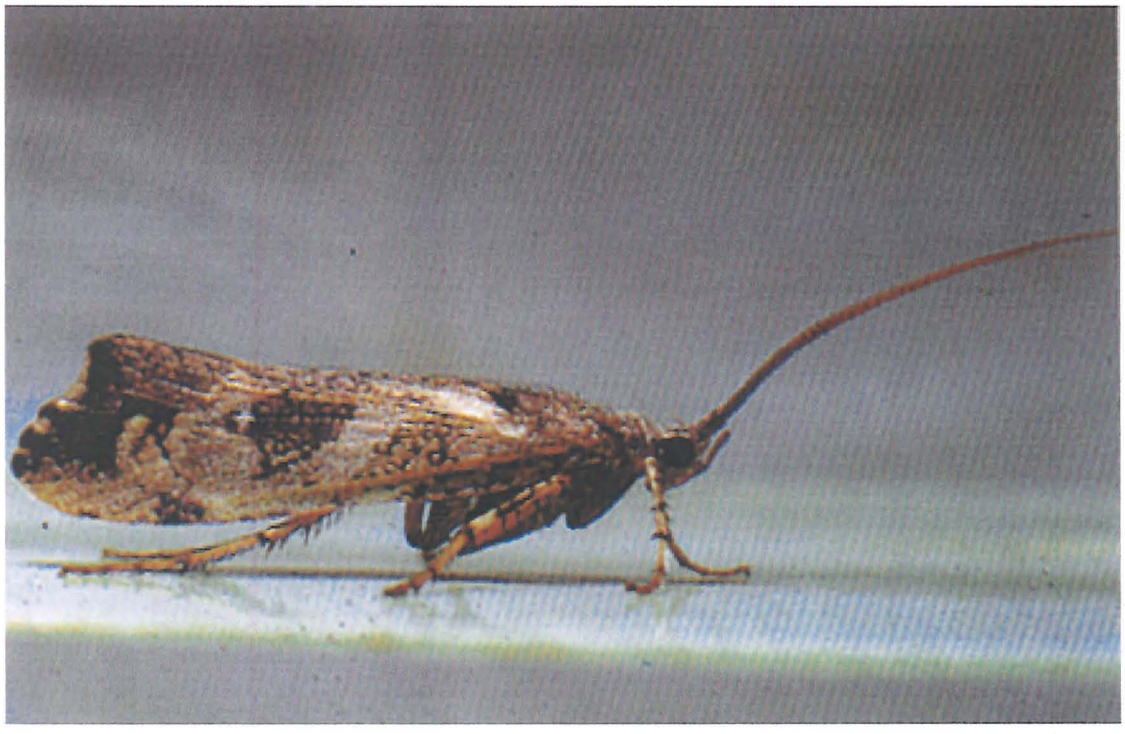
Chapter 3. Developmental trade-offs and life histories: strategic allocation of resources in caddis flies.



This chapter is an expanded version of Stevens, D.J., M.H. Hansell & P.Monaghan 2000 Developmental trade-offs and life-histories: strategic allocation of resources in caddis flies *Proc. R. Soc. Lond.* B **267**, 1511-1515.

3.1. INTRODUCTION

Trade-offs involving the partitioning of finite resources between developing body parts have important consequences for the evolution of both morphology and life history strategies (Trumbo 1999). The incorporation of resources into one structure precludes their allocation to another, thus determining the size and composition of different tissues and organs during growth (Stern & Emlen 1999). Additionally, for species that have more than one life-history stage, allocation decisions in one part of the life-cycle can also effect the amount of resources available to produce subsequent forms.

Many holometabolous insects, particularly butterfly species (Lepidopterans), have proved very useful in examining such developmental decisions (Gage 1995, Nijhout & Emlen 1998). Adult body structures grow from imaginal tissues after the cessation of larval feeding, using the resources accrued during the larval stage (Williams 1980). Adult feeding is very often minimal or non-existent, which means individuals are largely dependent on their larval reserves for reproduction. The majority of the adult body is made up of abdomen, thorax and wings. The abdomen consists almost entirely of the reproductive organs and reproductive reserves (Boggs 1981). Abdomen size in freshly emerged individuals therefore provides a good index of reproductive allocation (Boggs 1981). Somatic allocation is indicated by the resources in the rest of the body, which comprises mainly the thorax, and relative thorax mass is positively correlated with adult longevity (Karlsson & Wickman 1989).

Thus, not only does allocation to reproduction versus soma in such insects occur in a closed system (the pupa), it can readily be measured by looking at the relative sizes of thoraces and abdomens of emerging adults. Function and morphology are closely linked, and the designs of such organisms are known to be adjusted to their particular life-history strategies (Boggs 1981). Phylogenetic history will also determine to some extent the pattern of resource allocation during development (Stearns 1989), and individuals will also be constrained by developmental mechanisms (canalisation). However, to what extent morphological investment can be altered in line with predicted adult needs, albeit within some physiological limits, is still unclear. One way to test this is by manipulating the resource levels available at pupation, and examining the effect on resource allocation within the adult form.

Further, comparing the outcome of the same manipulation across closely related species likely to share the same developmental processes, but which differ in key aspects of their adult life-history, may help us to determine whether the observed outcome is fixed by developmental constraints or can be altered in line with strategic allocation decisions.

Caddis flies (Trichoptera) are closely related to the Lepidoptera and together they constitute the super-order Amphiesmenoptera (Morse 1997). The larvae are found in a wide-range of aquatic habitats, and many build a defensive case from particles of substrate held together with silk, a proteinaceous material (Wallace *et al.* 1990). Silk is a highly expressed protein and is relatively costly to synthesise (Craig *et al.* 1999). In a previous study (Chapter 2), I induced larvae of the case-building caddis fly *Odontocerum albicorne* to expend more silk. Larval food acquisition did not change, and thus the resources available at metamorphosis were reduced. The resultant adults had smaller wings and thoraces than control individuals, but abdomen size was maintained. The observed adult form could have arisen through constraints on pupal resource allocation pathways, possibly because the resources from the silk glands are 'committed' to the thorax area due to spatial or temporal proximity during development (Nijhout & Emlen 1998). Alternatively, given that adults of this species are very short lived and mate soon after emergence, the preservation of abdomen size may represent strategic preservation of potential fecundity at the expense of (post-reproductive) lifespan. If this is so, and resource allocation pathways in development are not fixed, I would expect that in a relatively long-lived caddis species, investment in the soma (thorax) would assume a greater importance. I would therefore predict that such a species, when faced with a depletion of larval resources, would be more likely to maintain thorax at the expense of abdomen size.

To test this prediction, I carried out a larval resource depletion experiment on *Glyphotaelius pellucidus*. Adults of this caddis species live for many months, with mating and egg laying occurring towards the end of this period (Svensson 1972). A significant reduction in allocation to the thorax could therefore have severe consequences in this species since being linked to longevity, this would increase the probability of death prior to reproduction.

3.2. METHODS

Glyphotaelius pellucidus larvae are found in lentic waters, many of which are temporary (Wallace *et al.* 1990). The larvae are almost exclusively herbivorous (Slack 1936). Their occurrence in ephemeral habitats is associated with a short larval-stage (seven months), and a relatively long adult-stage of four months, including an ovarian diapause (Svensson 1972). This is in contrast to the stream dwelling *O. albicorne*, which has a much longer larval stage (10 months), and an adult life-span of less than two weeks. Adult *G. pellucidus* can potentially take in liquids such as water or nectar (Crichton 1992), but observations of adult caddis flies feeding are rare.

The methods used with *O. albicorne* can be found in Chapter 2 and similar methods were used here. Forty eight, fifth-instar *G. pellucidus* larvae were collected on the 18th March 1998 from the margin of a small loch at the University Field Station, Rowardennan, Loch Lomond, Scotland where the experiment was also performed. All larvae were removed from their cases, blotted dry and weighed, before being randomly allocated to control and experimental groups. There was no difference in larval mass between the groups or sexes at this point (Fig. 3.1). Control larvae were placed back into their cases. The experimental larvae were not, thereby forming a case-rebuilding group. Rebuilding the larval case causes a significant increase in silk expenditure (Chapter 2). Each individual was placed into its own pot within a recirculating flume. Oak (*Quercus* sp.) leaves from the collection site were added to each rebuilder's pot for use as building material. Oak leaf litter is predominant where the larvae were collected, and it is utilised as both a food source and as the case material. Larvae do not feed whilst rebuilding, and once all experimental animals had rebuilt a new case, four squares (20mm sides) cut from oak leaves were provided as food for individuals of both groups. Food intake was recorded (so as to ascertain whether the manipulation affected resource acquisition) and eaten leaf squares were replaced every other day.

Larvae were considered to be in pupation once they had closed off the opening of their case with the characteristic silk 'sieve-membrane' (Hickin 1967). Pupae were placed into trays each covered by a net, and situated in the same flow-through tank. This procedure allowed the adults to be collected from each group upon emergence. Adults emerged overnight and were collected the next day. Individuals were killed by

freezing and were kept as such until the following measurements were taken: Fresh mass, forewing length, and both abdominal and thoracic dry mass and nitrogen content. Masses were measured on a Sartorius Supermicro balance to the nearest 0.0001mg and wing length was measured using a microscope with an ocular micrometer to the nearest 0.1mm. Thorax-abdomens were dried to constant mass in a drying oven at 60 ^{0}c and then separated. Nitrogen content of thoraces and abdomens was measured using a Carlo Erba 1106 elemental analyser.

All data were analysed using General Linear Models (GLM). Food intake values were measured using a $\log_{10}(x+1)$ transformation. To take into account any effect of body size, wing length and the mass of dry abdomens and dry thoraces were first regressed against fresh total body mass for the controls, and the residuals of both groups from this relationship were used in the GLM. To examine variation in proportional nitrogen content in the body parts, nitrogen contents were first regressed against the dry mass of the specific body part, and these residuals used in the analyses.

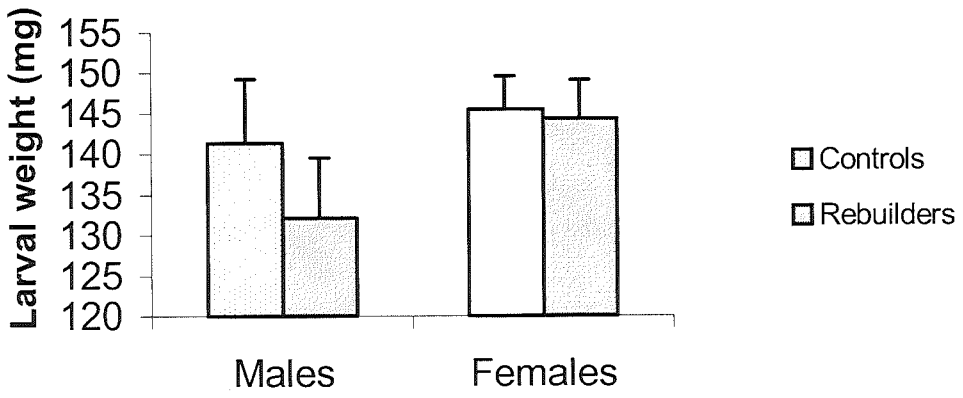


Fig. 3.1 Mean larval weights (with SE bars). Weights at the start of the experiment did not differ between sexes or groups (General Linear Model (GLM): Sex $F_{(1,47)} = 1.77, p > 0.05$; Group $F_{(1,47)} = 0.72, p > 0.05$; Sex x Group Interaction $F_{(1,47)} = 0.45, p > 0.05$).

3.3. RESULTS

There was no effect of the manipulation of larval resources on the mean number of leaf squares eaten per day by larval *G. pellucidus* although, in both controls and rebuilders, males consumed more than females over the course of the experiment (Fig. 3.2). While there were differences in the size of body parts between adult males and females, the pattern remained consistent in the control and rebuilding groups, and there was no interaction between sex and group for any of the analyses ($P > 0.41$). Figure 3.3 shows the effect of the manipulation on adult *G. pellucidus* morphology. There was no difference between the control and experimental groups in dry thorax mass, although females consistently had larger thoraces than males. Females also had longer wings. However, in contrast to thoraces, wings were affected by the manipulation, and were shorter in both sexes within the rebuilding group. Abdomen masses did not differ between the sexes, but like the wings, were relatively smaller in rebuilding individuals. Nitrogen composition of the tissues did not change significantly in either thoraces (Fig. 3.4a.) or abdomens, although male abdomens had a significantly greater percentage composition of nitrogen than females (Fig. 3.4b.).

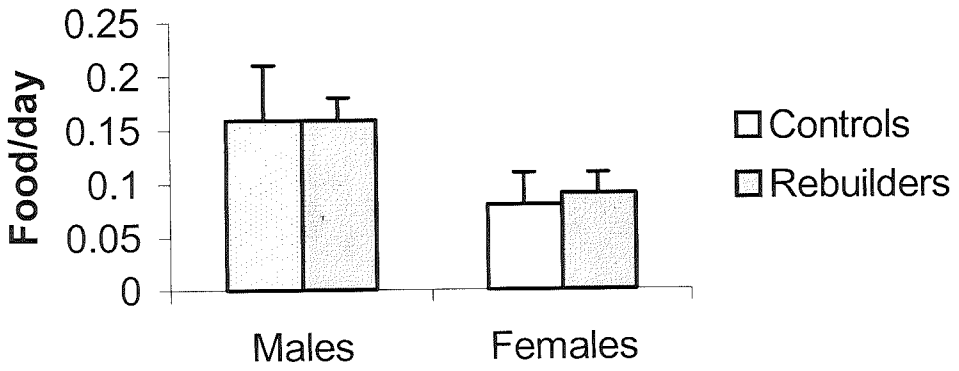


Fig. 3.2 Mean daily food intake (with SE bars). Rebuilding the larval case had no effect on average daily food intake, however, males consumed more than females (GLM: Sex $F_{(1,47)} = 5.33, p < 0.05$; Group $F_{(1,47)} = 0.15, p > 0.05$; Interaction $F_{(1,47)} = 0.00, p > 0.05$)

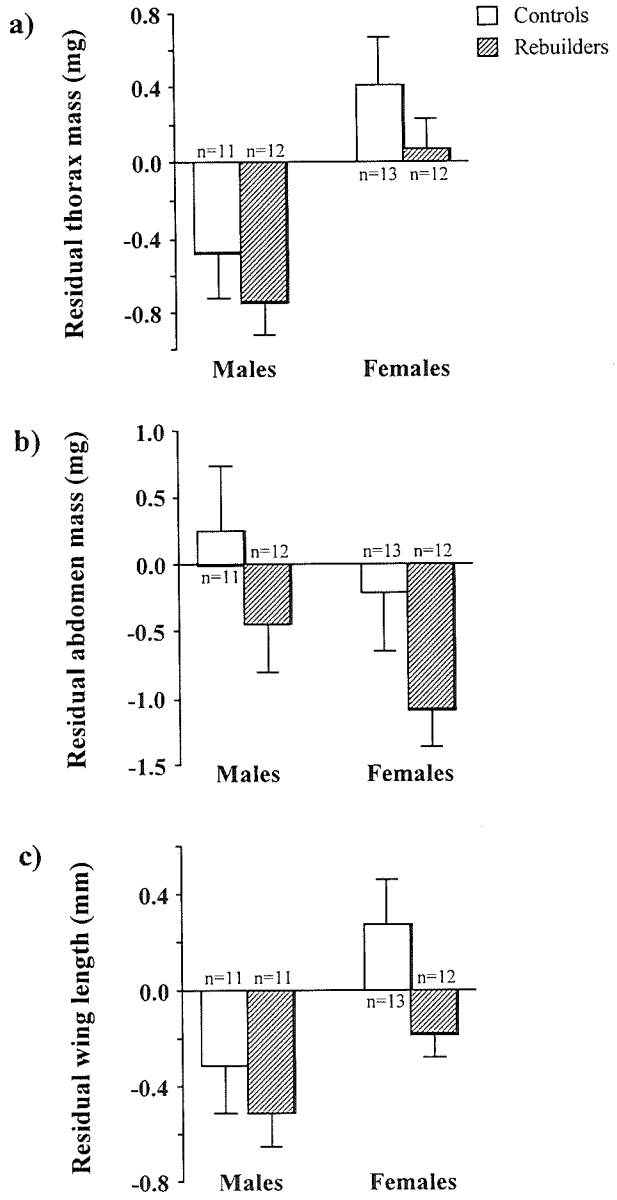


Fig. 3.3 Differences in adult morphology between groups: (a) dry thorax mass, (b) dry abdomen mass and (c) wing length. Data are presented as the mean residual values from regressions against wet total mass of the control group (± SE bars). Thorax mass differed between males and females but was not significantly different between control and experimental groups (GLM: Sex $F_{(1,47)} = 15.41, p < 0.001$; Group $F_{(1,47)} = 2.62, p > 0.10$). Abdomen mass was significantly lower in the rebuilders (GLM: Sex $F_{(1,47)} = 1.94, p > 0.10$; Group $F_{(1,47)} = 4.05, p < 0.05$) and their wings were shorter (GLM: Sex $F_{(1,46)} = 7.64, p < 0.01$; Group $F_{(1,46)} = 4.14, p < 0.05$).

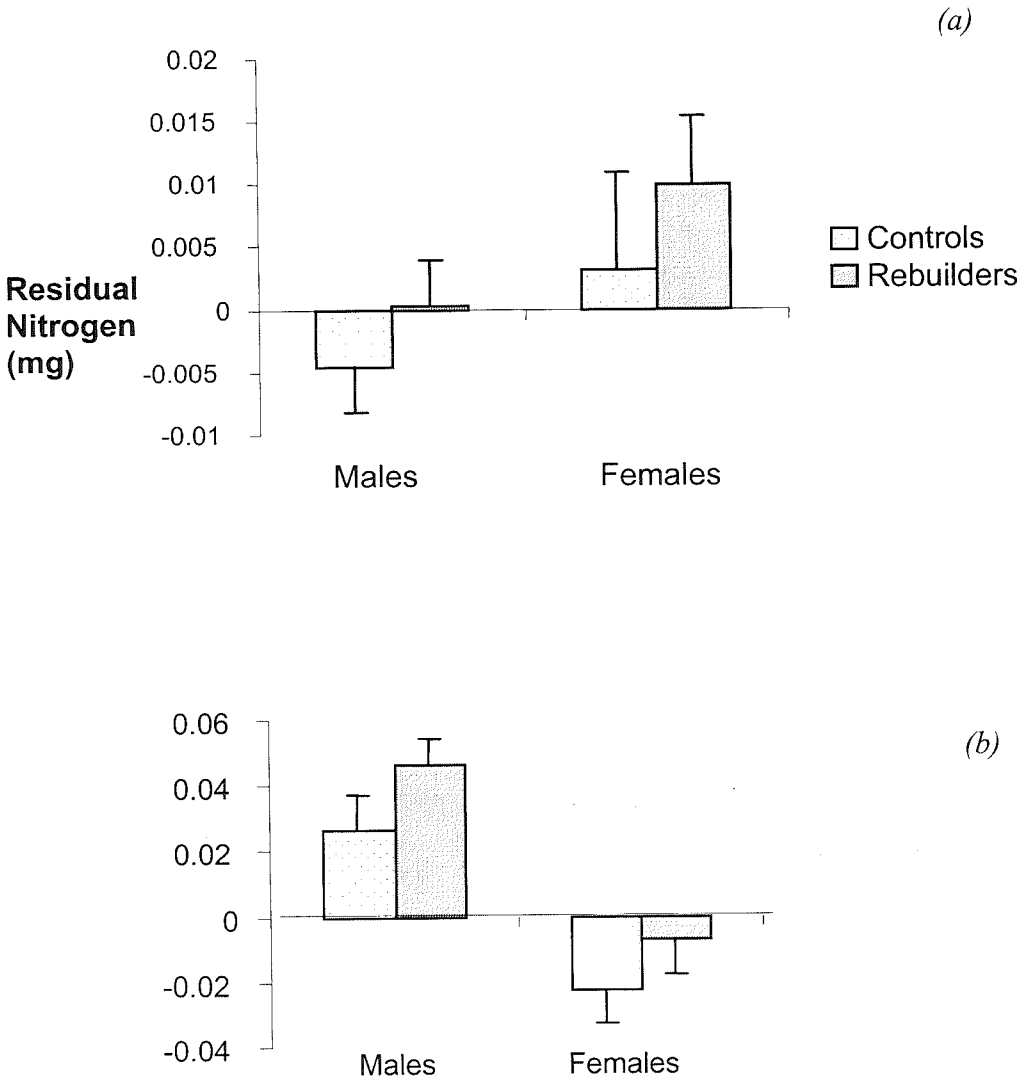


Fig. 3.4 Mean (a) thoracic nitrogen and (b) abdominal nitrogen content. Data are presented as mean residuals from the regression of nitrogen mass on either dry thoracic or abdominal mass (with SE bars). The proportion of nitrogen within the thorax did not differ between groups or sexes (GLM: Sex $F_{(1,46)} = 2.46$, $p > 0.05$; Group $F_{(1,46)} = 1.12$, $p > 0.05$). The proportion of nitrogen within the abdomen did not differ between groups, but males had a greater percentage composition than females (Sex $F_{(1,47)} = 26.10$, $p < 0.001$; Group $F_{(1,47)} = 3.15$, $p > 0.05$).

3.4. DISCUSSION

Silk production can be a considerable drain on resources for a number of invertebrates (Dudgeon 1987; Jakob 1991; Berenbaum 1993), and rebuilding of a new case by larval caddis flies has been found to cause a substantial increase in silk production (Chapter 2). When this occurs just prior to pupation, and is not accompanied by any compensatory increase in resource acquisition, it results in a reduction in the resources (particularly protein) available to build the adult form. In this study, increased larval expenditure of silk by fifth-instar larvae of *G. pellucidus* was not associated with any increase in larval feeding, and was observed to give rise to adults in which components of the body were reduced in size. These results provide further empirical support for a trade-off between larval defence and adult resource allocation, as also demonstrated via the same manipulation in the caddis fly *Odontocerum albicorne* (Chapter 2). However, the magnitude of the effect on different components of the adult form differed markedly between the two species. In *G. pellucidus* thorax size was relatively unaffected, in contrast to the reduced investment in abdomens and wings; in *O. albicorne*, the pattern was reversed, manipulated individuals tending to preserve their abdomens and reduce investment in their thoraces and wings (Fig. 3.5).

Competition between growing body parts for limited resources is expected on theoretical grounds (Nijhout & Wheeler 1996), but has been rarely demonstrated empirically (Trumbo 1999). Various mechanisms have also been proposed as to how this competition might create the allometric relationships that occur between developing body parts (Stern & Emlen 1999). However, the extent to which the outcome is a consequence of mechanistic developmental constraints, or free to be shaped by life history requirements, has remained unknown (Trumbo 1999). Following removal of the imaginal discs that give rise to the hindwings in the caterpillar *Precis coenia*, a compensatory increase in size was seen in the forewings, thorax and forelegs, but not the head or abdomen, of manipulated adults (Nijhout & Emlen 1998). Based on these results, the authors suggested that the 'partners' within such allocation trade-offs may be determined by their spatial or temporal proximity during development. That is to say, increased allocation to a particular body part will

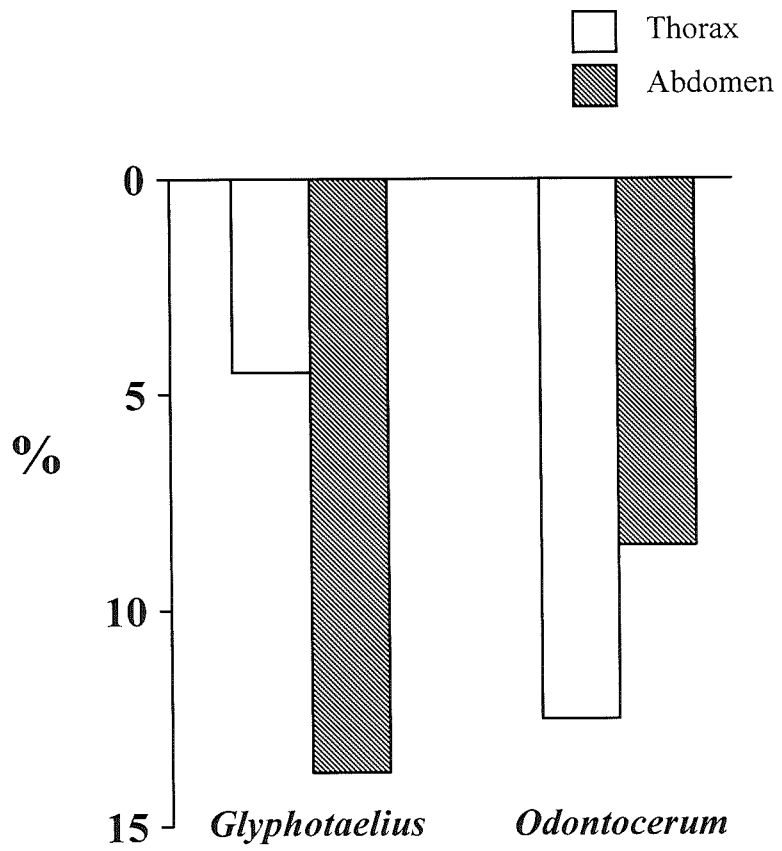


Fig. 3.5 The magnitude of the effect of depleted larval reserves on subsequent adult thorax or abdomen size in two caddis fly species with contrasting life histories. Data are combined for males and females, and presented as the percentage decrease in the mean mass of the rebuilding group compared to that of the respective control group. The thorax rather than the abdomen was most affected in *O. albicorne*, while the reverse was true in *G. pellucidus*. For details of *G. pellucidus* see text, and for details of *O. albicorne* see Chapter 2.

decrease the allocation to a body part that is developing nearby or at the same time. If constrained by such 'resource pools' during development, I would expect that the outcome of a particular depletion in closely related species would be the same. However, this is obviously not the case for the caddis flies studied here (Fig. 3.5).

The underlying physiological basis of metamorphosis and associated developmental pathways are likely to be very similar in these species (Odell 1998). Their life history patterns differ markedly however, and the pattern of resource allocation during metamorphosis appears to be directly in line with predicted adult requirements. Abdomen, and thereby reproductive investment, was preserved in the short-lived *O. albicorne*, at the expense of investment in the thorax, while the reverse was the case in the long-lived *G. pellucidus* (Figure 3.5). Preserving thoracic investment will maintain longevity (Karlsson & Wickman 1989; Gage 1995). This will be crucial in a species like *G. pellucidus* which has a very long adult life-span, considered to be an adaptation to the loss of suitable larval habitats during the summer (Svensson 1972). This species mates and lays eggs towards the end of summer, and thus survival over the relatively long period is vital for successful reproduction.

The observed reduction in abdominal allocation, is likely to be reflected in a reduced reproductive output in *G. pellucidus* when breeding does occur, since the abdomen comprises largely reproductive tissues. However, there are ways in which reproductive allocation could be improved during the relatively long adult life of *G. pellucidus*. Many insects hydrolyse flight muscle as they age to provide nitrogen for reproduction (Karlsson 1994) and females may also be able to incorporate nitrogenous resources obtained through matings into their reproductive reserves; such processes could redress reduced reproductive potential somewhat (Boggs 1990; Vahed 1998). This may well be the case here, as male abdomens have a greater nitrogen content than female abdomens, which could be due to protein-rich male spermatophores. If thoracic tissue cannot be added to after eclosion it may therefore be a better strategy to conserve somatic protein investment when faced with diminished resources during metamorphosis, if there is a chance of adding to reproductive reserves later on. Adults may also be able to obtain additional energy from nectar feeding, although little is known about such energy dynamics. In contrast, in *O. albicorne* the very brief adult life span, coupled with the fact that these adults emerge with their lifetime supply of

gametes already formed, means that maximising the gamete production potential of adults at eclosion is likely to be more important in maximising reproductive success.

There are probably various physiological, aerodynamic and phylogenetic constraints that keep relative investment in different body parts within viable limits. The observed effect of the reduction in resources on adult morphology appeared slightly more diffuse for *O. albicorne* (Figure 3.5). This may be a consequence of it expending more larval silk, as its sand grain case requires a greater investment compared to the organic case of *G. pellucidus* (Otto & Svensson 1980). There may be a point where investment in a body part cannot be reduced any further and still retain viability, and investment in other body parts then becomes affected. In both species, wing size was reduced to some extent, which may reflect a more generalised effect on the adult body. However, the extent to which this influences flight performance is unclear, since wing loading may have decreased due to the associated loss of body mass (Karlsson 1994). In any event, maintaining flight ability may be of lesser importance as most caddis fly species are reluctant fliers (Hickin 1967).

Clearly, the precise nature of developmental trade-offs will be influenced by a number of different factors. Most importantly, the results of this study on caddis flies demonstrate that depletion of resources available at pupation does not alter the adult body form in a fixed manner, as would be expected if this were entirely governed by developmental pathways. Rather, the way in which the adult body form is altered is flexible. My results suggest that this is in line with predictions based on life history theory, and a comparative study across a broad range of species could be used to examine this further.

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Chapter 4. The flight periods and ecology of adult Limnephilid caddis flies.

4.1. INTRODUCTION

Caddis flies (Order Trichoptera) are holometabolous insects, and almost all species have aquatic eggs, larvae and pupae, whilst the adults are terrestrial. The larvae inhabit a wide range of lentic and lotic habitats (Mackay & Wiggins 1979) where they usually make up a significant proportion of the benthos. They are one of the largest groups of aquatic insects (Wiggins 1987) and there is somewhere in the region of 10,000 extant species of Trichoptera worldwide, divided into 600 genera within 58 families (Morse 1997). There are nearly 200 species found in Great Britain (Wallace *et al.* 1990). There is some dispute as to the exact phylogenetic relationship, but it is generally considered that the Trichoptera and Lepidoptera are sister lineages (see Morse 1997). Like Lepidopteran larvae, caddis fly larvae also have the ability to produce silk from modified labial glands. This has allowed the diversification in feeding habits, defence and habitat selection that caddis fly larvae display. This diversity of habitats that larvae occupy is associated with a range of adult life-history traits.

Whilst the importance of caddis fly larvae to freshwater macroinvertebrate communities has been recognised for some time, and their biology relatively well studied (Wiggins 1987), the biology of the adult stages has largely been ignored (Halat & Resh 1997). As well as being generally nocturnal, adults are often small, inconspicuous and short lived, and this has probably adversely affected the amount of research carried out. Most information has come from trapping studies, with light traps proving the most effective way of collecting large numbers of individuals. With the exception of a few day active species, most adult caddis flies are attracted to light in a manner similar to other night flying insects (Crichton & Fisher 1978). This widespread behaviour has proved very useful for sampling techniques, especially as adult caddis flies are generally difficult to track successfully in the field. Many authors have employed light traps to determine the ecology of adults (Crichton 1971, Svennson 1972, Crichton & Fisher 1978 and references therein).

Svennson (1972) studied the flight periods and reproductive status of 27 Swedish species. Some species showed no peak of activity from their capture records, and these probably emerged throughout the summer, possibly due to there being more than one generation a year (see Chapter 6). Others showed a definite peak at the beginning of the flight period, which was associated with a synchronous emergence, whilst others showed a smaller peak at the beginning and the main peak at the end of the flight period. This pattern was generally found in the longer lived species that had a synchronous emergence, but the main period of activity (mating and/or oviposition) was towards the end on their life, after an ovarian diapause (see below). Crichton (1971) devised a classification system for these different flight periods: (1) an extended flight period with diapause, from spring to autumn, (2) a short flight period with no diapause in spring and summer, and (3) a short flight period with no diapause in the autumn. This was subsequently revised by Sommerhäuser *et al.* (1997) who added a fourth category for non-diapausing species that have a short flight period in spring.

The Limnephilidae is the largest family of caddis flies found in Britain – 58 species in 21 genera (Wallace *et al.* 1990). Limnephilids are generally large species and the adults readily come to light traps, sometimes some distance from the larval habitat (Svensson 1974). The occurrence of the larvae of some species in ephemeral habitats means they often have a long adult stage (up to five months in some species) and Limnephilids exhibit the greatest range of life history strategies of any of the British Trichopteran families. Novak & Sehnal (1963) first described the ovarian diapause undergone by many Limnephilids that emerge in the spring. They developed a classification of four developmental stages that females pass through ranging from the immature female (a) to the female after oviposition (d). Early emerging species do so in stage (a), whilst species emerging in late summer/autumn have passed through this stage during the larval or pupal stage, and emerge in stage (b) (the maturing female) or (c) (the mature female before oviposition). A photoperiod of 12 h during the 5^{th} instar was found to lead to ovarian diapause, whereas an 18 h photoperiod lead to direct development for *L. rhombicus* (Denis 1977). This may explain why individuals from species that normally diapause in central Europe do not undergo diapause at higher latitiudes (Gíslason 1977).

However, diapause is also absent from populations at high altitiudes (Novak & Sehnal 1963, , Hiley 1977) and this may be a temperature related effect.

The captures of Limnephilids from the Rowardennan light trap have been documented previously as part of the Rothampsted Insect Survey (Crichton 1971) but they have never been considered solely in any detail. In fact, Crichton (1971) reported that only nine Limnephilids were collected from this trap in 1968 out of 133 Trichopteran adults in total, which seems a surprisingly low number for a light trap situated next to Britain's largest area of freshwater. The flight periods quoted for British Trichoptera are generally from southern English populations, but such species have a slightly different timing in Scotland (Crichton & Fisher 1978), such that Scottish Limnephilid populations emerging in spring are later than those further south and the flight period of autumn species are earlier (Crichton 1971, Richardson 1991). The species caught by this trap are also interesting because it is near to lentic water bodies, whereas most traps have usually been operated near streams. This chapter describes the flight periods of the Limnephilid species caught in the trap, and relates them to what is known of their general ecology. Subsequent chapters will examine the dynamics of resource use in these different species (Chapter 5) and how resource allocation patterns relate to life history strategies (Chapter 7).

4.2. METHODS

Adult Limnephilid caddis flies were collected from the light trap at the University Field Station, Rowardennan (OS grid reference: NS378958) between the 7th April and the 16th October 1997, the 20th March and 28th November 1998 and the 27th March and 3rd December 1999. The light trap is situated in mixed deciduous woodland and is in close proximity to a number of water bodies. It is approximately 100 metres from Loch Lomond and 150 metres from the Dubh Loch. There are also a few small streams and temporary pools within the vicinity. Details of the type of trap can be found in Williams (1948), and it is operated for the purposes of providing Macrolepidoptera to the Rothamsted Insect Survey. It basically consists of a 200 W bulb that provides the light that insects are initially attracted towards. Once in the trap sloping glass sheets lead the insects into a blacked out killing jar, which is lined with plaster of Paris that has been impregnated with tetrachloroethane. Catches from Monday – Thursday nights were removed daily, whilst the Friday – Sunday nights catches were combined. Each catch was frozen upon removal from the trap. Capture date was recorded as Julian day, whilst for the combined weekend catches, the Saturday date was used. Defrosted individuals were identified to species and sexed using Macan (1972) and Mclachlan (1880).For the purposes of constructing flight periods for each species, daily catches were pooled into weeks.

4.3. RESULTS & DISCUSSION

Table 4.1 gives the species collected in this study together with the list of species collected from the same trap in 1968 (Crichton 1971) and the species list of larvae from the Loch Lomond catchment (Adams *et al.* 1990). Crichton (1971) records only 6 species from the light trap, whilst the larval check-list mentions 10 species that could possibly occur within the vicinity of the light trap (Adams *et al.* 1990). Only two species (*Glyphotaelius pellucidus* and *Limnephilus marmoratus*) were recorded by all three studies and 11 out of these 25 different species were only recorded by this survey. Neither source claims to be a complete record, and this study is therefore the most comprehensive survey yet of the Limnephilid species found in this area.

4.3.1. Numbers

Over the 28-week period during 1997, 431 adults were collected; 367 individuals were collected over the 37 weeks in 1998 and 578 in 37 weeks in 1999. Fig. 4.1. shows the total weekly catches for each year. In 1997, the greatest numbers of individuals were caught in October (201), with the greatest weekly catch in the week which spanned September & October (week 40 – 110 individuals). For 1998, October was again the month with the highest catch (92) but the largest weekly catch (50) was week 14, in April, and was made up entirely of *Apatania wallengreni* (see below). The highest catch for 1999 was 203 in September, and the highest weekly catch (82) was also in September. Most of this inter-year variation can probably be explained by nightly weather conditions, particularly temperature. Thus, a total of 1376 individuals comprising 20 species from 11 genera were collected from the light trap over the three years. Table 4.2a-c. shows these species listed chronologically based on the date of their first capture in 1997. Generally speaking, the species collected were consistent between years, however *L. griseus* and *L. flavicornis* were only caught in 1997, and *Allogamus* auricollis was only collected in 1999. Of the total number collected, 76.5% were males, and the species specific sex ratios varied from 25% to 100% male (Table 4.3). The low total in 1998 was mainly due to proportionally fewer females being caught that year (9%) compared to 14 & 17% in

the other two years. Light traps generally tend to attract more males anyway, but distance from the hatching area can affect this too (Svensson 1972). The nearer the trap to the emergence site, the more females are caught, which implies a difference in flight ability between the sexes (see Chapter 7).

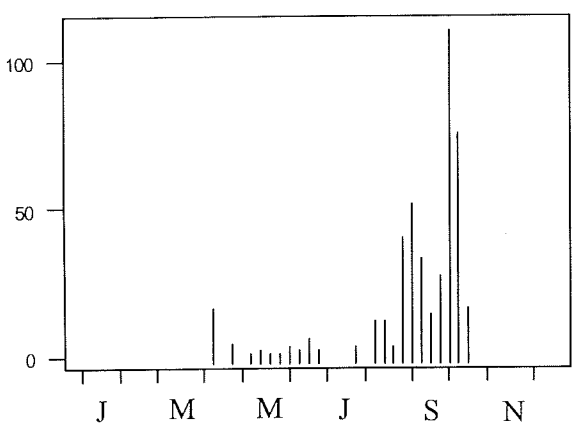
4.3.2. Flight periods

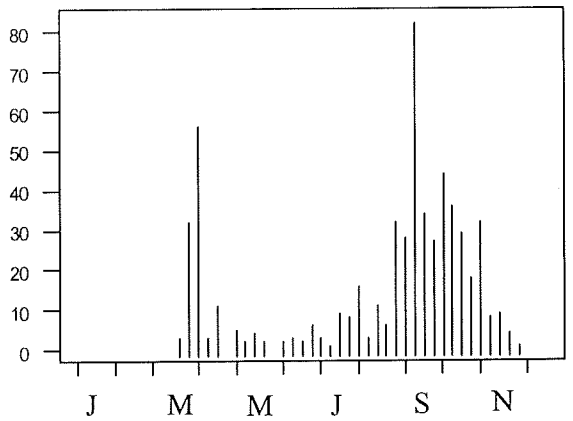
Fig.4.2a-i summarises the flight periods of the 9 most abundant species from the light trap. Despite the modest sample sizes, they do demonstrate the range of flight periods exhibited by the Limnephilidae (Crichton 1971), and generally agree with the results of Svensson (1972). The classification system of Sommerhäuser *et al.* (1997) for flight periods has been adopted here (see Table 4.4 for details). The sampling period probably started just after the first emergence of *A. wallengreni*, Fig. 4.2a shows the typical pattern for a species with a brief synchronous emergence, and short adult life span (Type A). There is an initial peak in numbers in late March/early April, which gradually declines, the whole period lasting about a month. This is shorter than the published flight period that is reported as lasting till June. The other extreme of flight period types (Type C) can be seen in *Glyphotaelius pellucidus* (Fig. 4.2b). Here there is an initial small peak in May when individuals emerge (see Chapter 3), but then none are caught again until August after the ovarian diapause, with the peak capture in September. Svensson (1972) operating light traps in Southern Sweden, found a similar pattern, but with the highest captures slightly earlier, in August. The increase in activity in the autumn coincides with mating and egg laying behaviours (Svensson 1972). Adults are known to emerge over a period of about a month in April and live for about 4-5 months in captivity (see Chapters 3 & 5). Therefore all individuals caught are from the same cohort which emerged at roughly the same time.

Micropterna lateralis (Fig. 4.2c.) also emerges in May from temporary water bodies, but does not undergo an ovarian diapause (Type B) (Crichton 1971, Svensson 1972). This strategy is unusual, and *M. lateralis* is the only species caught to display it. Adults appear to mate quite soon after emergence (Svensson 1972), and individuals are not caught later than early August, so adult life span is probably about

Table 4.1. Comparison of the Limnephilid species recovered from the Rowardennan light trap between 1997-1999, with captures from the same trap in 1968, and a checklist of larval forms found within the vicinity of the University Field Station where the trap is situated.

	Light trap '97-'99	Crichton (1971)	Adams *et al.* (1990)
Species			
Allogamus auricollis	✓		
Anabolia nervosa	✓		✓
Apatania wallengreni	✓	✓	
Chaetopteryx villosa	✓		✓
Glyphotaelius pellucidus	✓	✓	✓
Halesus digitatus	✓		✓
Halesus radiatus	✓		✓
Limnephilus auricula	✓		
Limnephilus affinus			✓
Limnephilus bipunctatus		✓	
Limnephilus centralis	✓	✓	
Limenphilus decipens			✓
Limnephilus flavicornis	✓		
Limnephilus griseus	✓		
Limnephilus lunatus	✓		
Limnephilus luridus	✓		
Limnephilus marmoratus	✓	✓	✓
Limnephilus nigriceps	✓		
Limnephilus rhombicus			✓
Limnephilus sparsus	✓		
Mesophylax impunctatus	✓		
Micropterna lateralis	✓		
Micropterna sequax		✓	
Potomophylax latipennis	✓		✓
Stenophylax vibex	✓		
Total	20	6	10





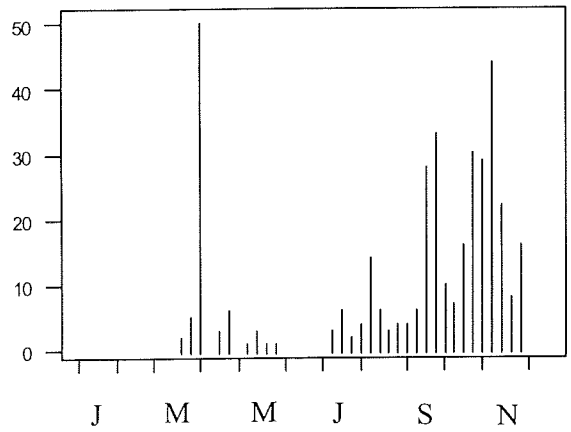


Fig. 4.1. Total weekly catches of adult Limnephilids from the Rowardennan light trap 1997-1999.

Table 4.2a, b & c. The first and last capture dates, and total numbers of males and females of Limnephilid caddis fly species collected from the University Field Station light trap between (a) 7th April - 16th October 1997, (b) 20th March - 28th November 1998 and (c) 27th March – 3rd December 1999. Species are listed by the 1997 order of capture. Dates in bold are for species where the earliest capture was female.

	MALES			FEMALES		
Species	Date of first capture	Date of last capture	n	Date of first capture	Date of last capture	n
Apatania wallengreni	7th April	26th April	20	-	-	0
Glyphotaelius pellucidus	13th May	27th September	33	27th August	8th September	7
Micropterna lateralis	17th May	10th August	12	-	-	0
Limnephilus luridus	16th June	10th August	5	**10th June**		1
Limnephilus centralis	26th June	25th August	7	-	-	0
Limnephilus marmoratus	6th August	4th October	24	10th August	11th October	9
Limnephilus sparsus	10th August	27th September	25	10th August	4th October	8
Mesophylax impunctatus	14th September	27th September	3	**12th August**	9th October	5
Limnephilus lunatus	14th August	8th October	94	21st August	7th October	40
Stenophylax vibex	25th August	30th September	9	9th September	-	1
Limnephilus griseus[1]	26th August	30th August	2	-	-	0
Limnephilus auricula	30th August	13th September	7	-	-	0
Potomophylax latipennis	3rd September	-	1	**30th August**	27th September	3
Halesus radiatus	4th September	16th October	57	9th September	11th October	19
Anabolia nervosa	4th October	16th October	15	**27thSeptember**	16th October	8
Limnephilus flavicornis[1]	27th September	-	1	27th September	-	1
Limnephilus nigriceps	27th September	-	1	-	-	0
Halesus digitatus	27th September	30th September	2	30th September	-	1
Chaetopteryx villosa	4th October	16th October	8	11th October	13th October	2

Table 4.2b. 1998

	MALES			FEMALES		
Species	Date of first capture	Date of last capture	n	Date of first capture	Date of last capture	n
Apatania wallengreni	20th March	30th April	63	29th March	16th April	6
Glyphotaelius pellucidus	7th May	30th September	16	11th May	13th May	3
Micropterna lateralis	19th May	5th August	7	-	-	0
Limnephilus luridus	14th July	10th August	8	18th July	-	1
Limnephilus centralis	6th July	23rd September	6	-	-	0
Limnephilus marmoratus	16th July	26th October	13	2nd August	-	1
Limnephilus sparsus	27th August	7th October	23	**24th May**	7th September	3
Mesophylax impunctatus	-	-	-	-	-	-
Limnephilus lunatus	19th August	28th November	59	26th September	20th November	7
Stenophylax vibex	13th August	26th September	16	-	-	0
Limnephilus griseus[1]	-	-	-	-	-	-
Limnephilus auricula	4th August	-	1	-	-	-
Potomophylax latipennis	5th September	-	1	-	-	-
Halesus radiatus	17th September	26th October	22	**29th August**	7th November	3
Anabolia nervosa	23rd September	22nd October	10	21st October	-	1
Limnephilus flavicornis[1]	-	-	-	-	-	-
Limnephilus nigriceps	-	-	-	-	-	-
Halesus digitatus	26th September	16th October	3	26th September	-	1
Chaetopteryx villosa	30th September	27th November	85	3rd November	25th November	8

Table 4.2c. 1999

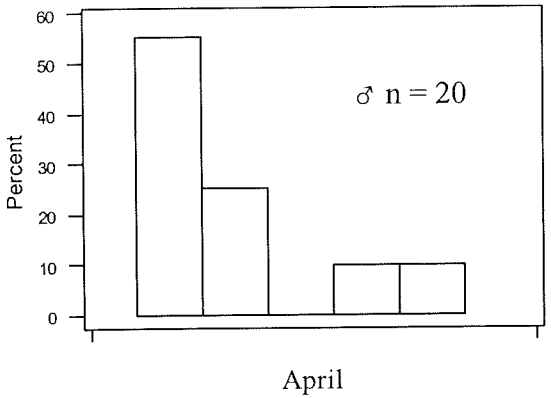
	MALES			FEMALES		
Species	Date of first capture	Date of last capture	n	Date of first capture	Date of last capture	n
Apatania wallengreni	27th March	24th April	104	3rd April	-	1
Glyphotaelius pellucidus	8th May	18th September	14	8th May	18th September	6
Micropterna lateralis	12th May	30th July	14	-	-	0
Limnephilus luridus	28th July	13th August	6	**19th June**	24th July	5
Limnephilus centralis	10th June	11th September	16	**21st May**	27th May	8
Limnephilus marmoratus	5th August	9th October	18	5th August	27th September	8
Limnephilus sparsus	16th June	27th September	19	24th July	18th September	15
Mesophylax impunctatus	7th October	-	1	**22nd September**	7th Ocotber	2
Limnephilus lunatus	16th August	2nd November	70	9th September	2nd November	38
Stenophylax vibex	13th August	18th September	21	1st September	4th September	3
Limnephilus griseus[1]	-	-	-	-	-	-
Limnephilus auricula	-	-	-	-	-	-
Potomophylax latipennis	4th September	6th October	11	6th September	15th September	5
Halesus radiatus	11th September	19th October	48	**7th August**	13th November	39
Anabolia nervosa	18th September	21st October	19	**15th September**	15th Ocotber	9
Limnephilus flavicornis[1]	-	-	-	-	-	-
Limnephilus nigriceps	-	-	-	6th August	-	1
Halesus digitatus	11th October	2nd November	2	-	-	-
Chaetopteryx villosa	30th September	3rd December	56	**19th October**	13th November	18
Allogamus auricollis[2]	28th September	-	1	-	-	-

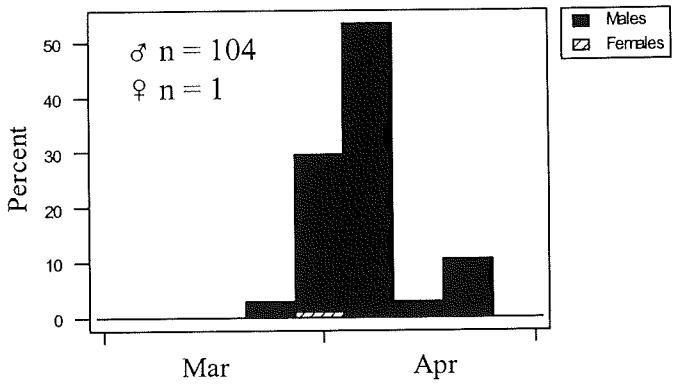
[1] These species were only caught in 1997
[2] This species was only caught in 1999

Table 4.3. Sex ratios of adult Limnephilids collected from the light trap 1997-99 and the same values from the national Rothamsted Insect Survey 1964-1968 (Crichton 1971). Values are % males.

Species	1997	1998	1999	Crichton (1971)
Allogamus auricollis	-	-	*	
Anabolia nervosa	65	91	68	83
Apatania wallengreni	100	91	99	
Chaetopteryx villosa	80	91	76	81
Glyphotaelius pellucidus	83	84	70	86
Halesus digitatus	67	75	100	76
Halesus radiatus	75	88	55	76
Limnephilus auricula	100	*	-	
Limnephilus centralis	100	100	89	89
Limnephilus flavicornis	*	-	-	
Limnephilus griseus	100	-	-	
Limnephilus lunatus	70	89	65	68
Limnephilus luridus	83	89	55	82
Limnephilus marmoratus	73	93	69	57
Limnephilus nigriceps	*	-	^	
Limnephilus sparsus	75	88	56	78
Mesophylax impunctatus	38	-	25	
Micropterna lateralis	100	100	100	90
Potomophylax latipennis	25	50	69	96
Stenophylax vibex	90	100	88	88
Overall	76	91	73	72

- None caught
* Only one male caught
^ Only one female caught





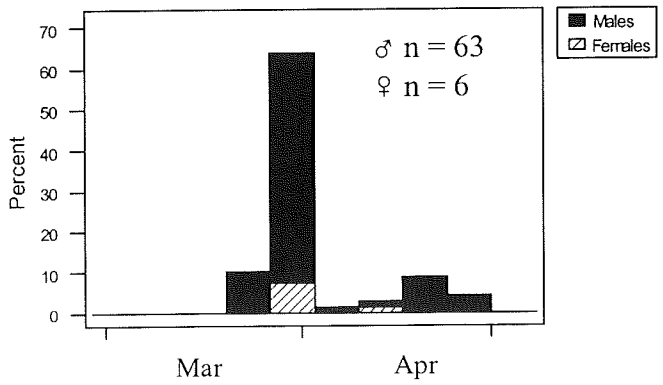
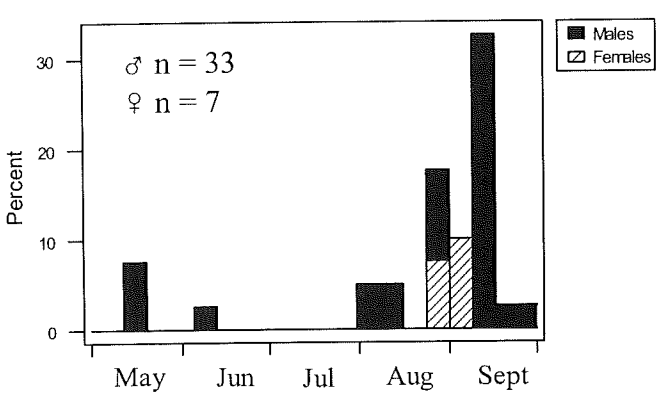
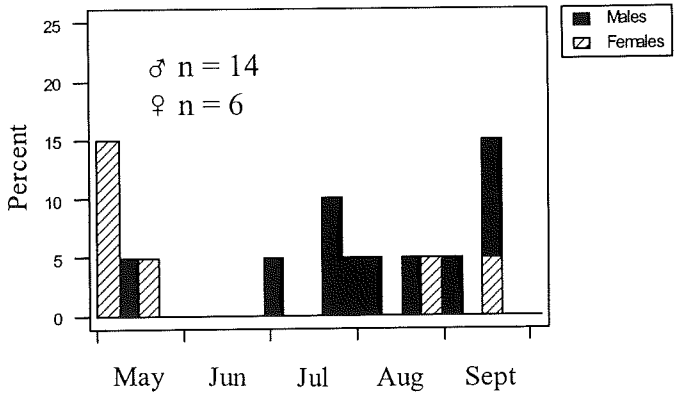


Fig. 4.2.a-i. The flight periods of the nine most abundant species of Limnephilids caught with the Rowardennan light trap, 1997-1999. Data are presented in weekly totals expressed as a percentage of total number caught for that species during the year.

Fig.4.2a *Apatania wallengreni*





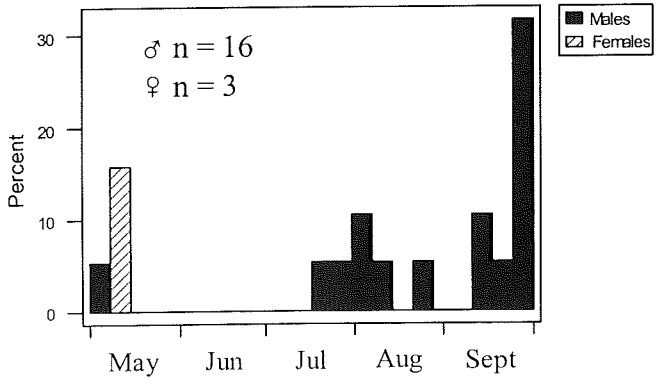
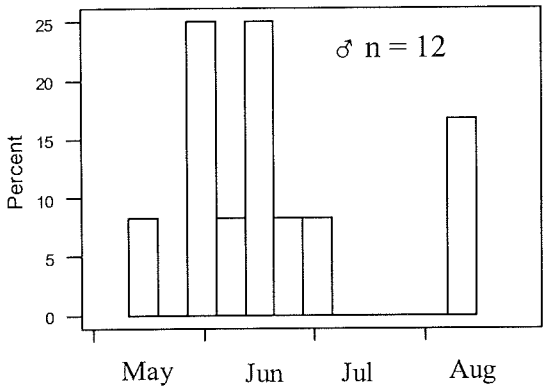
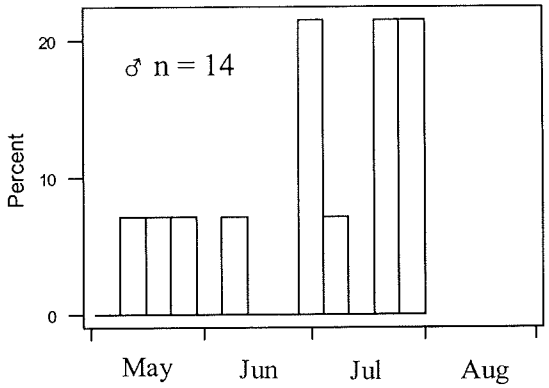


Fig. 4.2 b. *Glyphotaelius pellucidus*





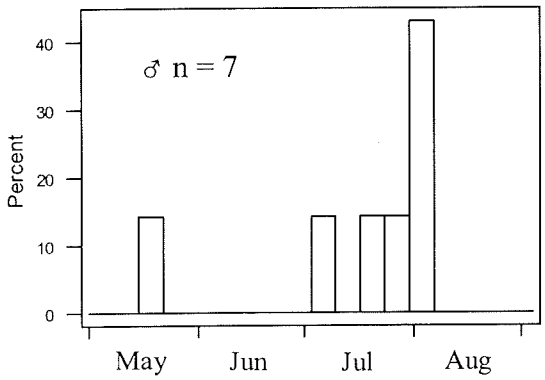
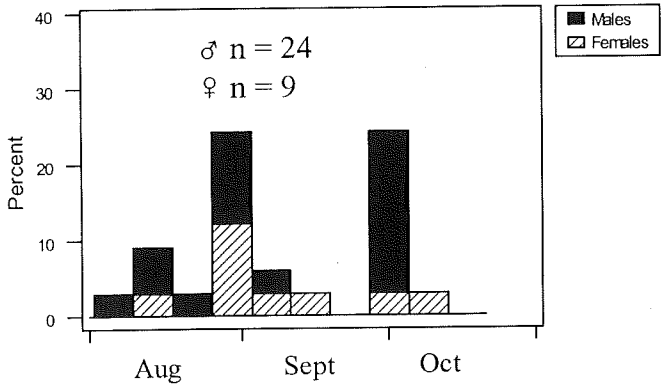
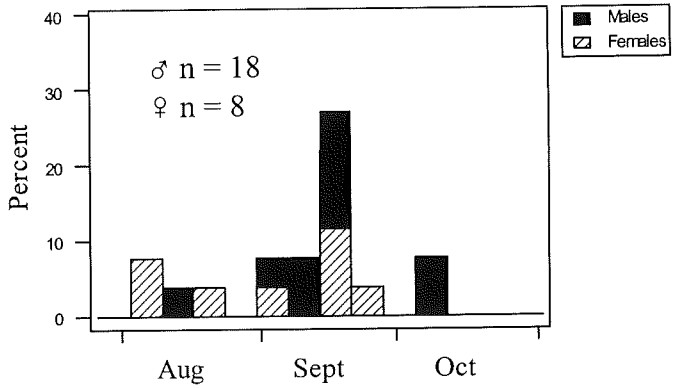


Fig. 4.2c. *Micropterna lateralis*





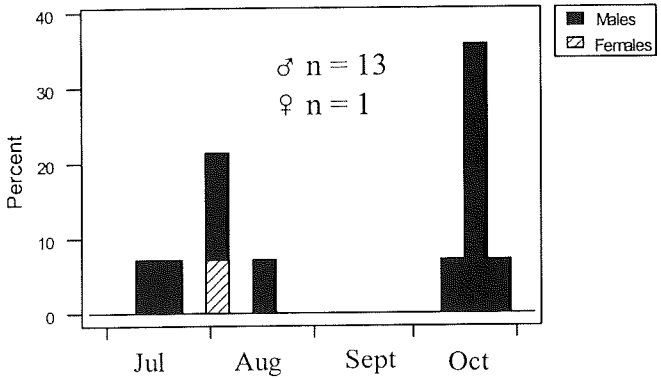
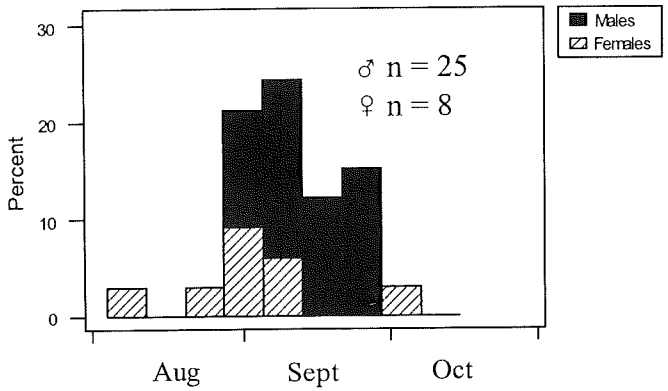
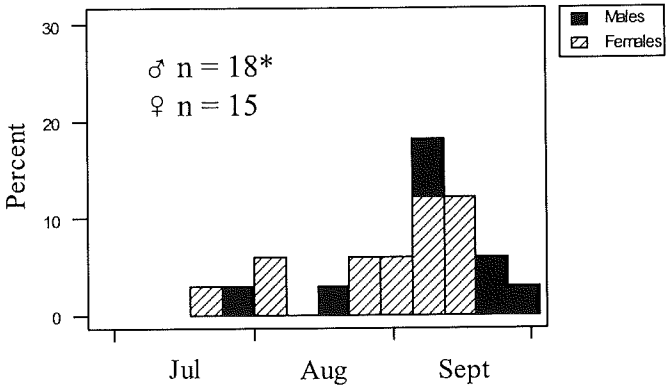
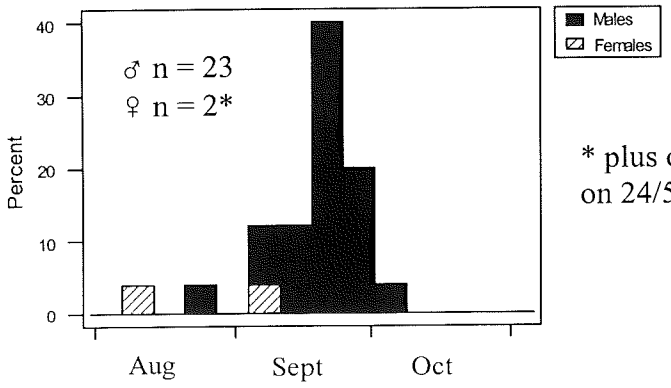


Fig. 4.2d. *Limnephilus marmoratus*



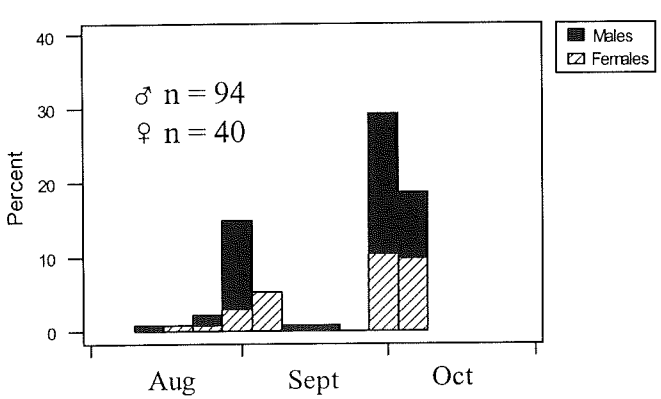


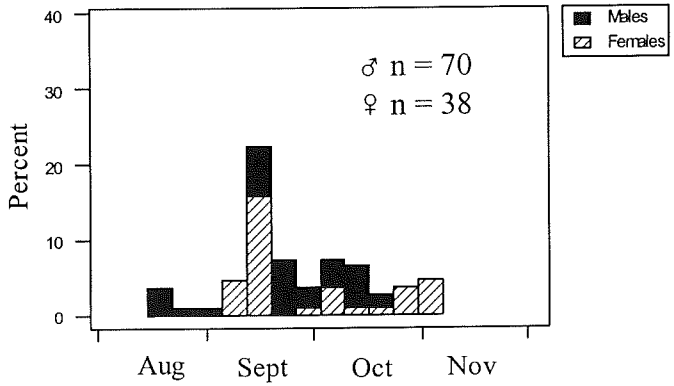
* plus one male
caught on 16/6/98



* plus one female caught
on 24/5/98

Fig. 4.2e. *Limnephilus sparsus*





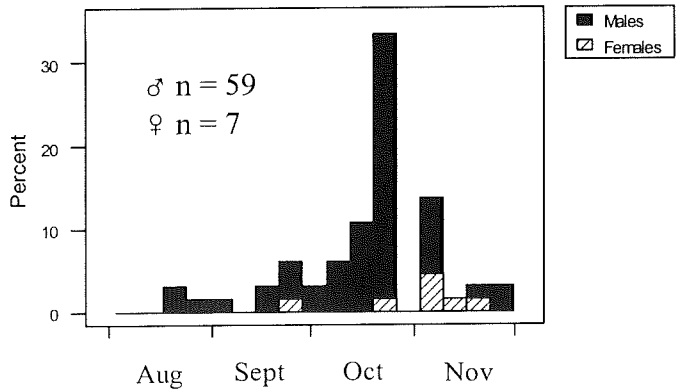
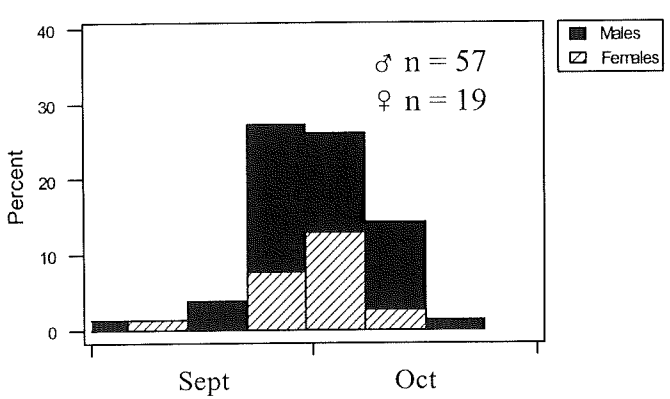


Fig. 4.2f. *Limnephilus lunatus*



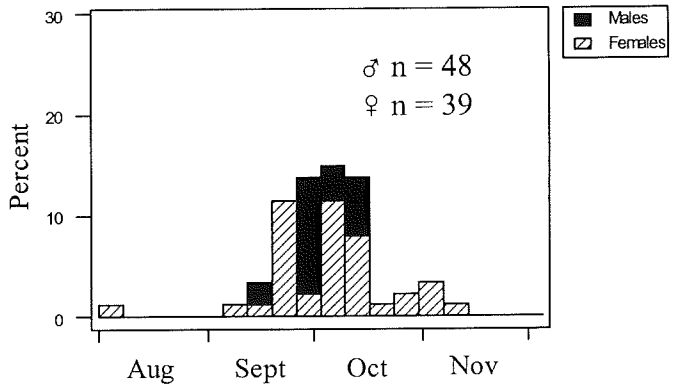
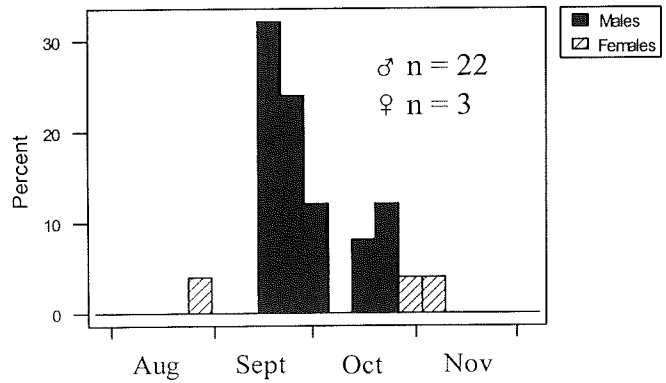
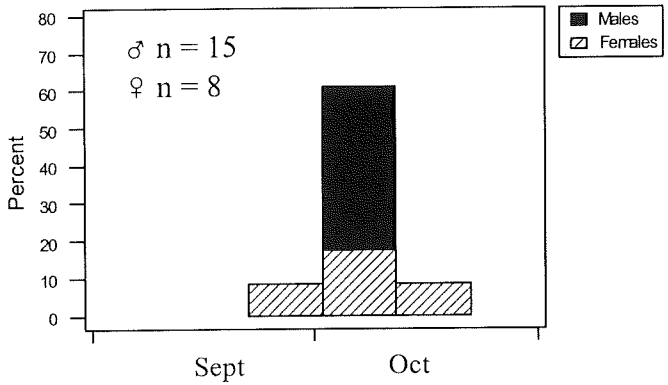
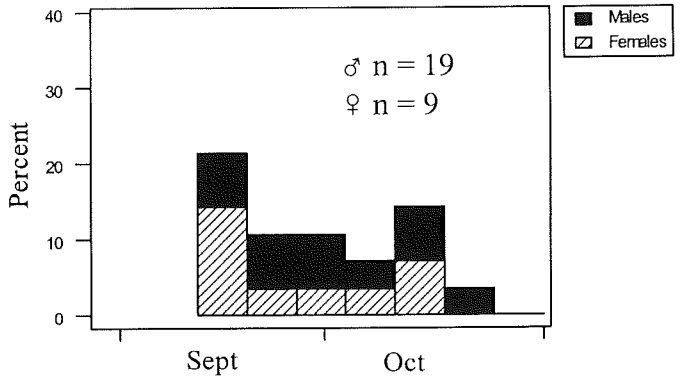


Fig. 4.2g. *Halesus radiatus*







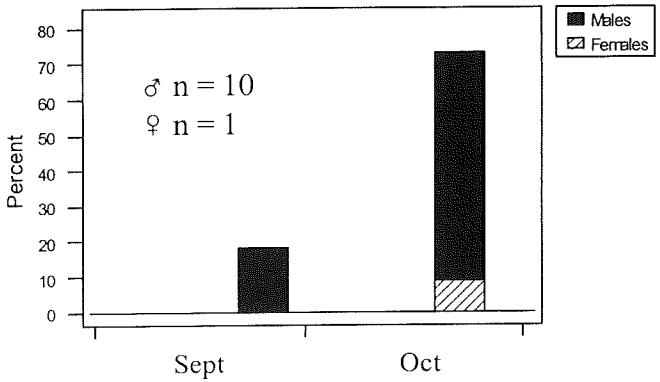
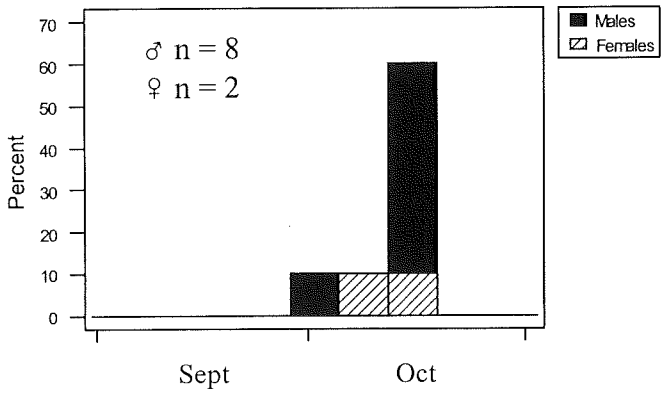
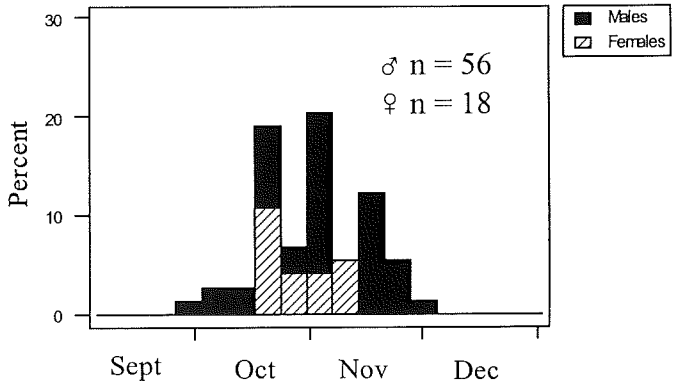


Fig. 4.2h. *Anabolia nervosa*





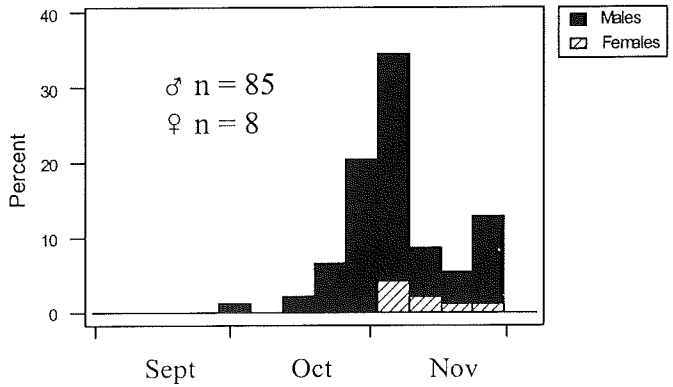


Fig. 4.2i. *Chaetopteryx villosa*

Table 4.4. The ecology of the species recovered from the light trap. Numerals refer to quarter of the month.

Species	Larval Habitat [a]	Flight Period	Flight Period (literature)	Type [b]
Apatania wallengreni	Stony shores of medium-large lakes. Scotland & Lake district	III March – IV April	April - June[1]	A
Glyphotaelius pellucidus	Ponds & lake margins that dry up in summer. Uncommon in Scotland.	I May- IV September	II May - IV October[2]	C
Micropterna lateralis	(Woodland) streams & ditches that dry up in summer.	II May- II August	IV June - II July[2]	B
Limnephilus luridus	Acidic (woodland) pools that dry up in summer.	II June – II August	I June – IV August[2]	C
Limnephilus centralis	Pools & ponds that dry up in summer.	II June – III September	I June – IV August[2]	C
Limnephilus marmoratus	Most lentic (inc. temp.) waters. Common in Scotland	III July – III October	I July – IV October[2]	C
Limnephilus sparsus	Puddles & pools that dry up in summer	III May – I October	I June – IV October[2]	C
Mesophylax impunctatus	Permanent lake margins. Found in Scotland only north of the Forth/Clyde line.	II August – I October	May – November[1]	B
Limnephilus lunatus	Most permanent waters. Very common species	II August – IV November	IV May – II November[2]	C
Stenophylax vibex	Temporary streams, similar habitats to *M. lateralis*	II August – IV September	May – October[3]	C
Limnephilus griseus	Acidic pools that dry up in summer	III August – IV August	II September – III October[2]	C
Limnephilus auricula	Temporary pools & ditches	I August – II September	I May – IV October[2]	C
Potomophylax latipennis	Streams, rivers & stony lake shores in Scotland.	IV August – I October	I July – IV September[2]	D
Halesus radiatus	Streams, rivers & lake shores.	I August – II November	I September – I November[2]	D
Anabolia nervosa	Rivers, lakes & large ponds with mineral substrate.	III September – III October	July – November[1]	D
Limnephilus flavicornis	Most lentic (inc. temp.) waters. Uncommon in Scotland.	III September	I June – I November[2]	C
Limnephilus nigriceps	Lakes & large ponds in Scotland & N. England.	I August – III September	September – October[1]	D
Halesus digitatus	(Woodland) streams & rivers	IV September – I November	I September – II November[2]	D
Chaetopteryx villosa	Streams, rivers & small lakes	IV September – I December	October – November[1]	D
Allogamus auricollis	Stony rivers & large streams. Common in Scotland.	IV September	-	D

[a] after Wallace (1991)

[b] after Sommerhäuser *et al.* (1997)

[1] from Macan (1973)

[2] from Hickin (1967)

[3] from Crichton (1971)

A – Short flight period in spring, no diapause

B – Short flight per. spring & summer, no diapause

C – Extended flight period spring/summer to autumn, with a diapause

D – Short flight period autumn, no diapause

two months. It most likely lays its eggs under damp logs or stones, and the eggs will quiesce until the larval habitat reappears in the autumn.

The three *Limnephilus* species show a similar pattern of flight (all Type C). These species, as do most *Limnephilus* species, undergo an ovarian diapause (Novak & Sehnal 1963). *L. marmoratus,* and *L. lunatus* (Fig. 4.2d&f) were first caught in August, and the last catches were in October. *L. sparsus* (Fig. 4.2e.) however, probably starts to emerge slightly earlier in June as Svensson (1972) found, with mating first occurring in August, compared to September for *L. lunatus*. The flight period of *L. sparsus* also ends earlier than the other two *Limnephilus* species. The peak captures for these two species however, as with *G. pellucidus*, was slightly earlier in the Swedish study. Svensson (1972) did not catch any *L. marmoratus*. The published flight period for *L. lunatus* starts from May, but catches in Scotland do not seem to start until August (this study & Crichton 1971) as is similar in Sweden (Svensson 1972), and the same is also true for *S. vibex*. Crichton (1971) speculated that, within Britain, the Scottish populations of some species may not exhibit an adult diapause, and this warrants further investigation. In *L. sparsus* there is some evidence of females having a flight period that starts earlier than that of the males (Fig. 4.2.e), which was also observed by Svensson (1972).

Halesus radiatus and *Anabolia nervosa* (Figs. 4.2g&h) have relatively short flight periods for Limnephillid species (although the end of sampling in 1997 truncated this), and both show a peak in numbers at the end of September/beginning of October (Type D). The flight period is superficially similar to the spring flight period of *A. wallengreni*, however adults of that species are probably much shorter lived and emergence is more synchronous. *H. radiatus* emerges with well developed ovaries, and adult lifespan is less than a month (Svensson 1972). *A. nervosa* is also probably very similar (Crichton 1971). *C. villosa* (Fig. 4.2i.) is an unusual species in that adults are still in flight in December, even in Scotland (Crichton 1971).

Table 4.4. summarises the larval habitat, and the flight periods of all the Limnephilids recovered from the light trap. It also compares published records of flight periods with those from this study. *A. wallengreni, M. impunctatus, P. latipennis, A. nervosa, L. nigriceps, L. lunatus, H. radiatus, C. villosa* and *A. auricolla* probably emerged from Loch Lomond, whilst *G. pellucidus, L.*

marmoratus and *L. flavicornis* probably emerged from the Dubh Loch. Of the other species, most would have emerged from temporary water bodies within the woodland around the light trap, which is why they were not recorded in the invertebrate survey of the Loch Lomond catchment (Adams *et al.* 1990).

4.3.3. Comparisons with other studies

The flight periods and sex ratios generally agreed with accounts published elsewhere (Crichton 1971, Svensson 1972, Sommerhäuser 1997). There were some exceptions: *A. wallengreni* was only caught until late April, whilst it has previously been recorded as being still in flight in June, and no *L. bipunctatus* were collected, despite there being records from this trap previously (Crichton 1971). Some of the more abundant species (*L. lunatus, L. marmoratus, L. sparsus, H. radiatus*) collected in this study were similarly common in comparison with the national Rothamsted Insect Survey (Crichton 1971). Other species that were collected in reasonable numbers here were uncommon in that survey (*A. wallengreni, C. villosa, G. pellucidus*) and some nationally common species (*H. digitatus, A. auricollis*) were uncommon in the Rowardennan light trap. Interestingly, the only *A. wallengreni* caught in the Rothamsted survey came from the Rowardennan trap, but the records for that trap were only for 1968 and were classified as 'incomplete' (Crichton 1971) as can be seen by the collection of only nine Limnephilids that year. These differences between this trap and the national survey reflect differences in species distributions, but also differences in trap placement, and this demonstrates the need to examine light trap records in conjunction with larval sampling. Light trap catches are heavily dependent on their placement in relation to larval habitats (Svensson 1974), as well as nightly weather conditions. Actual flight periods (i.e. emergence) may not differ with latitude as has been suggested, but low night time temperature in Scotland may instead affect flight activity, which could produce similar results. The adults of some species are also day active, or not attracted to light. Larval sampling however can often miss species that occur in temporary habitats (see Adams *et al.* 1990), and so any comprehensive faunal survey must include both light trapping and larval collecting.

4.3.4. Life histories

In general the pattern of captures of adult caddis flies from this trap reflect the range of life history strategies employed by Limnephilid caddis flies from lentic waters. Those from temporary water bodies can either spend the period when the larval habitat is dry as diapausing adults, or lay their desiccation-resistant eggs in damp places; the eggs will then develop once the suitable habitat is restored (Novak & Sehnal 1963). In turn this affects the flight period, with species in the former category having long flight periods with very few captures during the summer diapausing period. The individual strategy should influence whether they invest their resources mainly into survival, or more into the production of drought resistant eggs. Adults from some of the smaller diapausing species are known to mate prior to the diapause (e.g. *L. griseus, L. auricula, L. vittatus*) and females possibly incorporate resources from the male spermatophore into somatic functions (Svensson 1972). Species from more permanent water bodies have much shorter adult stages, but temporal food limitation in the larval stages means that certain instars often diapause as well. This means they can avoid periods of food shortage, and also control the timing of adult emergence and thus synchronise the hatching of their subsequent offspring with certain environmental conditions. Most of these species lay their eggs in autumn, but some are spring species, and this is probably related to the types of food resources utilised by the larval form (Otto 1981).

Whilst caddis flies can be difficult to investigate, particularly in the adult stage, light trap catches provide a useful resource. If individuals are preserved correctly after collection, they can be analysed to provide information on mating history (Svensson 1972), resource allocation (Chapter 5), feeding history (Petersson & Hasselrot 1994) and even age (Petersson 1989). Light trap catches have previously mainly been used to determine species distribution, but can potentially provide much more information on a taxa which is still poorly understood.

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Chapter 5. Adult resource dynamics.

5.1. INTRODUCTION

Resource allocation patterns and life history strategies are known to be affected by both the timing and the nature of an organism's resource acquisition (Boggs 1992, Cuthill & Houston 1997). In animals with complex life cycles, some stages may not feed, or have a diet consisting of only certain nutrients. Resources accrued in previous life stages are therefore very important, and their allocation during body reorganisation at metamorphosis will be adjusted to the organisms future requirements (Boggs 1981). These allocation commitments at metamorphosis will constrain life history traits, as investment in one will limit investment in others (Roff 1992, Stearns 1992). For instance, a long life will demand a greater somatic investment at the expense of allocation of these resources to reproduction (Bell & Koufopanou 1986, Lessells 1991). In such organisms, the levels of these stored resources will inevitably decrease over time as they are utilised. The rate of decline is likely to be organ or tissue specific, and will also depend on the type of activities being undertaken. Therefore, both the initial amount of allocation and the rate of utilisation will limit life history traits (Calow 1979). Investment in many body parts will be fixed during development, such that they cannot subsequently be altered (Karlsson & Wickman 1989). However, individuals may be 'phenotypically flexible' (Piersma & Lindström 1997), and resource allocation may to some extent be a dynamic process, which allows individuals to move resources between certain body parts or functions during their life. This way they could maximise investment in different traits at particular times, or in response to certain circumstances (Perrin & Sibly 1993).

In holometabolous insects, the adult form is built during metamorphosis using the resources liberated from the breakdown of the larval body. In species with limited adult feeding (e.g. Lepidoptera and Trichoptera) the proportion allocated to reproduction will be determined by the expected reproductive output – in monandrous species females invest relatively more in reproduction than females of polyandrous species, whereas males show the reverse pattern (Karlsson 1995). For species with long life spans, there is also a need to invest more in self-maintenance (Karlsson &

Wickman 1989). Trade-offs will therefore arise during development in the allocation of larval resources to adult life history traits.

It has generally been considered for such holometabolous species that the resources contained within the abdomen at eclosion are the reproductive reserves and those in the rest of the body the soma (Boggs 1981). Many butterfly species display mass losses over their adult lifetimes, usually from the abdomen (Karlsson 1987) and this can be viewed as mainly reproductive output. In species where the adult diet is lacking nitrogenous compounds, the prior expenditure of abdominal reproductive reserves would be expected to limit future reproduction. Recent evidence suggests however, that many such species can also break down flight muscle and use the nitrogen liberated from this for reproduction (Karlsson 1994, 1998, Stjernholm & Karlsson 2000) as has been previously demonstrated in many hemimetabolous insects (see Zera & Denno 1997 for review). Such lifetime decreases in thoracic mass seem to be a common phenomenon in females, but may only occur in males from species that are polyandrous due to an increased investment in spermatophore production (Stjernholm & Karlsson 2000). Somatic and reproductive reserves therefore differ because when the adult diet is lacking nitrogen muscle mass cannot be added to, whereas abdominal stores could be increased through breaking down muscle mass and, for females, through nuptial gifts. Accordingly, the resources invested in the thorax potentially have an influence on many life history traits.

Thorax size is positively associated with longevity (Karlsson & Wickman 1989, Gage 1995), and flight ability (Srygley & Chai 1990, Srygley & Kingsolver 1998), and as suggested above it may also act as a source of reproductive nutrients. Whether the transfer of resources from the thorax to the abdomen is obligate or facultatively determined by requirements (e.g. number of matings) is unknown. There is some evidence that male nuptial gifts may in some way stimulate an increase in the breakdown of female thoraces in the butterfly *Pieris napi* (Karlsson 1998). It may be that thoracic resources initially contribute towards soma and flight ability, and are transferred to the abdomen once reserves there are becoming depleted. A decrease in abdominal mass may mean thoracic reserves could be re-allocated without losing flight ability (Kingsolver & Srygley 2000), and the costs of self-maintenance may also decrease when flight muscle is histolysed (Mole & Zera 1993, 1994; Tanaka 1993).

Mass losses through resource expenditure such as those demonstrated in the Lepidoptera are also likely to occur in adult Trichopterans, as they are closely related to Lepidopterans (Morse 1997). Svensson (1975) reported a 40% decline in dry body mass over the season for males of the caddis fly *Potomophylax cingulatus*, which he assumed to be reproductive losses and depletion of fat reserves. Adult caddis flies also lack nitrogen in their diet, and so the use of the thorax material for reproduction may also occur. There is also a range of life spans and mating systems, and so species may vary in their use of body resources. Species differ in the importance they place on thoracic and abdominal reserves when faced with nutritional deficiencies at metamorphosis, and this may be a result of the way they utilise the body resources during their lifetime (Angelo & Slansky 1984). *Odontocerum albicorne*, a short-lived caddis fly species which emerges with fully developed eggs and is probably monandrous, sacrificed adult thoracic mass when manipulated to expend extra larval protein on case building (Chapter 2). The longer-lived caddis fly species *Glyphotaelius pellucidus*, which passes through an ovarian diapause, reacted to having less larval reserves at pupation by emerging with a smaller abdomen (Chapter 3). These patterns may be related to the dynamics of adult resource use. Allocating resources preferentially to the abdomen may be a better tactic when thoracic resources are not used in reproduction, whilst maintaining thoracic reserves may be related to their use initially in soma, and then subsequently for reproduction.

We would therefore expect adult caddis flies to also display mass losses from the abdomen and/or thorax during their lifetimes, and that the pattern of resource use will depend on the life-history strategy of the species. To determine how the resources within the body are utilised, adult Limnephilid caddis flies were collected in a light trap and the mass of thorax and abdomen measured. Individuals collected from this light trap came from a range of species, which differ in their ecology, particularly life span. For all the species sampled, the emergence period (i.e. the period over which adults are eclosing) is relatively short compared to the overall flight period (i.e. the period when adults are in flight, which includes the emergence period), and so collection date is a rough approximation of age.

Many arthropods show a negative relationship between size and maturation date (Higgins 2000 and references therein), and so structural body size was also

recorded to control for body size effects. As well as recording the mass, muscle (thoraces) and fat (abdomen) were removed to determine which resources in particular were responsible for any changes in mass for the light-trapped individuals. For instance, if after the removal of muscle, the remaining thorax exoskeleton showed a similar decline to overall thorax mass, then thoracic mass losses were not just due to loss of muscle mass, but some other compounds such as lipids or substances within the cuticle. Similarly with fat, if lean abdomen mass shows a decline over time, this can be attributed to reproductive losses occurring in the abdomen. Wing loading and thorax: abdomen ratio (both measures of potential flight ability) were also calculated for all individuals.

To determine whether mass lost from the thorax was obligate or dependent on matings, some species (inlcuding the non-Limnephilid *O. albicorne*) were reared from pupae and similar measurements taken upon death of the adult. This allowed measurement of resource use by individuals of known age kept within a constant environment. The thoraces and abdomens of captive reared animals were analysed for nitrogen content to determine whether its quantity changes within the thoraces or abdomens of individuals despite a lack of reproductive output.

5.2. METHODS

The individuals collected from the light trap in 1997 (see Chapter 4) were used for the examination of changes with age in the wild. Wet mass was measured to the nearest 0.01mg and right fore-wing length to the nearest 0.1mm using a dissecting microscope with an occular micrometer, or for individuals with wings longer than 18mm, a pair of dial callipers was used instead. Wings, legs and the head were then removed, and the remaining thorax-abdomen was dried for three days in an oven at 60°C. These were then separated, weighed to the nearest 1μg, and then frozen. Once all individuals were processed in this way, a number were selected for flight muscle and fat content analysis. Samples of individuals were taken from the light trap catches so as to give a wide selection of species and sampling dates, as well as to give (where possible) approximately equal numbers of the sexes within each species.

5.2.1. Flight muscle and fat contents

The method to digest flight muscle is adapted from Petersson (1995) and, for abdomen lipids the methods of Jacobsen & Sand-Jensen (1994) were used. Dried thoraces and abdomens from individuals selected for analysis were re-dried at 60° c for 24 hours. Individual body parts were weighed and then each placed in a 0.5ml eppindorf tube. Each tube was filled with either 30% Potassium Hydroxide solution (thoraces) or Diethyl Ether (abdomens), and left for 24 hours. They were then centrifuged for 10 minutes at 13,000 rpm, and the supernatant decanted. The tubes were then filled for the second time with the appropriate reagent, left for 24 hours and centrifuged as before. After removing the supernatant, distilled water was added, and the tubes centrifuged again. For the thoraces this was repeated twice more. Thoraces and abdomens were again dried for 24 hours at 60° c and re-weighed.

5.2.2. Captive rearing

Throughout the spring, summer and autumn of 1999, caddis fly pupae and some larvae were collected from a range of freshwater bodies situated in Scotland and Northern England so as to facilitate the collection of newly emerged adults from a number of different species. Although many larval populations were identified, most yielded small numbers of pupae and/or subsequent adults. The collection of larval stages was generally avoided because of the problems of maintaining animals and also because laboratory rearing may influence resource acquisition/allocation. Many sites only had low densities, and quite often on visits to known populations, no animals could be found, which indicated that individuals moved to specific pupation sites. Pupae were transferred into plastic beakers (568ml), that were two thirds full of water. Each of these pots was covered with a net and aerated via an airline with an air stone (any larvae collected were kept in the laboratory until they entered pupation and then transferred). Between three and ten pupae were placed in each pot. The pots were kept in a constant temperature room, which had a photoperiod and temperature regime that was adjusted every two weeks to be ambient. Emerging pupae were able to crawl right out of the water on 'ladders' made from strips of rigid plastic mesh, where they would shed their pupal skin. Even when relatively large numbers of pupae were collected, often only a fraction successfully developed into adults. Despite sample sizes being generally too small to look at temporal variation within species, enough animals were collected to give information on resource allocation, which could be compared inter-specifically along with data from light trapped specimens (Chapter 7).

Adults generally emerged at night, and were collected the next morning. For each species, emerging individuals were alternately either killed by freezing, or transferred to their own plastic beaker (568 ml) that was again covered with netting, but did not contain water. Adults in the latter category were provided with sugar solution on a regular basis and kept until they died, upon which time they were also frozen. This method meant that not only resource allocation at emergence, but also life span and associated changes in body mass could be recorded for each species (where sample size allowed). Individuals were treated in the same way as the light trapped individuals, except that nitrogen analysis was performed on the dried thoraces and abdomens using a Carlo Erba 1106.

For data from the light trap, all body size, body mass and resource allocation data were log transformed prior to regression against capture date (Julian Days). Data for males and females were analysed separately. Mass and nitrogen data from the captive reared individuals was analysed with general linear models (GLM), using sex as a factor and life span as covariate. To adjust for the number of tests performed, the sequential Bonferroni technique (Holm 1979) was applied to statistical tests performed within each sex of each species. As suggested by Wright (1992) and Chandler (1995), the critical value was set to 10% prior to Bonferroni adjustments.

5.3. RESULTS

Most of the individuals collected from the light trap were male (see Chapter 4) and as such, unless otherwise stated all results refer to males only. Seven species were represented sufficiently to allow analysis for changes in body mass, and three of these (*Limnephilus marmoratus, Limnephilus lunatus & Halesus radiatus*) also provided enough females to examine. No species displayed a change in wing length over time (Table 5.2, $p > 0.104$), and so at any one sampling point, same-species individuals were assumed to be roughly the same age.

Changes in the masses of male thoraces and abdomens over the course of the flight periods showed species specific patterns (Tables 5.1 & Fig. 5.1). Three species (*Glyphotaelius pellucidus, Micropterna lateralis, & L. lunatus*) all displayed significant mass losses from both thorax and abdomen over the course of their flight period. *Apatania wallengreni* males only lost mass from their abdomens (although individuals were not caught over the entire flight period for this species). *L. marmoratus, L. sparsus* and *H. radiatus* did not demonstrate any significant mass changes. Females of *L. marmoratus* and *H. radiatus* lost mass from their thoraces, whilst female *L. lunatus* showed no significant losses (Table 5.1, Fig. 5.2).

5.3.1. Thorax-abdomen ratio and wing loading

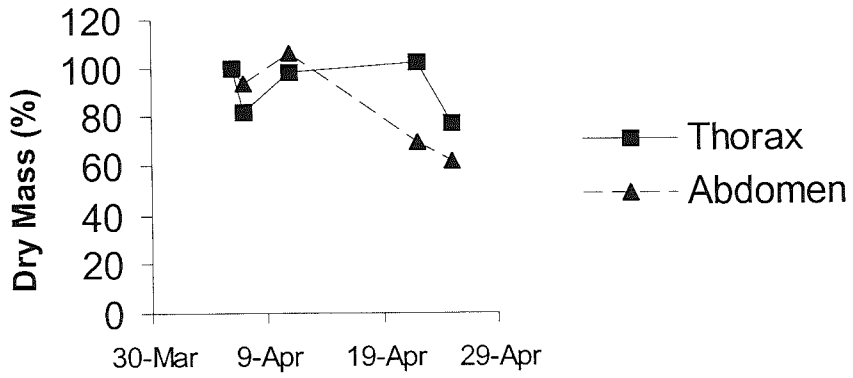
Wing loading (dry thorax & abdomen mass/wing length^2) decreased in all species where both thorax and abdomen mass were lost (Table 5.2.). It also decreased in female *L.lunatus*, although these individuals only lost mass from their abdomens. No other species displayed a significant change in wing loading during their flight period.

The ratio of thorax mass to abdomen mass (TAR) also changed over the course of the flight periods (Table 5.2). Of the three species that displayed a significant decline in the mass of both structures, none maintained its TAR. *A. wallengreni* was the only other species to display a change in TAR. In all cases where TAR changed, it increased.

Table 5.1 Mean thoracic and abdominal masses for adult Limnephilid caddis fly species caught using a light trap. Data are pooled into groups on the basis of capture date. Values are dry masses in mg with standard errors. Data are for males unless otherwise stated.

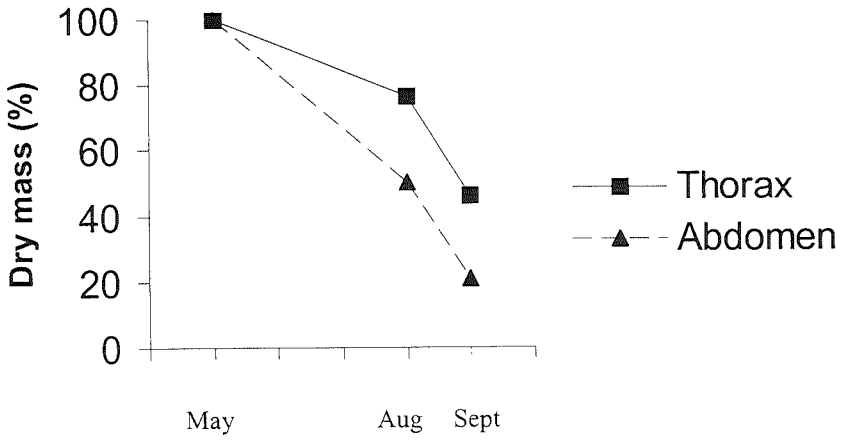
	Date	*n*	Thorax	s.e.	Abdomen	s.e.
Apatania wallengreni ♂	6-Apr	10	0.836	0.059	0.329	0.018
	7-Apr	1	0.682	-	0.307	-
	11-Apr	5	0.822	0.024	0.349	0.062
	22-Apr	2	0.854	0.041	0.229	0.009
	25-Apr	2	0.647	0.024	0.202	0.042
Glyphotaelius pellucidus ♂	May	4	7.480	1.01	7.380	1.12
	August	7	5.732	0.39	3.706	0.48
	September	22	3.441	0.13	1.550	0.10
Micropterna lateralis ♂	May	2	5.550	0.58	5.101	0.88
	June	8	4.400	0.32	3.532	0.50
	August	2	2.739	0.14	1.471	0.01
Limnephilus marmoratus ♂	August	13	4.124	0.332	2.742	0.504
	September	10	3.968	0.207	2.720	0.408
	October	1	3.282	-	2.530	-
Limnephilus sparsus ♂	26-Aug	6	1.849	0.176	1.186	0.235
	2-Sep	5	1.214	0.145	0.592	0.085
	9-Sep	5	1.512	0.281	0.625	0.165
	16-Sep	4	1.899	0.343	1.412	0.338
	23-Sep	5	1.400	0.248	0.778	0.102
Limnephilus lunatus ♂	August	25	3.189	0.192	3.081	0.250
	September	38	2.442	0.089	1.791	0.171
	October	31	2.255	0.082	1.097	0.103
Halesus radiatus ♂	6-Sep	2	5.980	1.870	5.730	1.700
	20-Sep	3	5.810	0.651	4.801	0.717
	4-Oct	47	4.993	0.197	3.625	0.301
	18-Oct	5	4.388	0.272	3.246	0.668
L. marmoratus ♀	August	5	6.892	0.370	8.061	0.477
	September	3	3.907	0.912	3.105	0.579
	October	1	5.1878	-	8.5763	-
L. lunatus ♀	August	6	3.212	0.270	4.45	1.22
	September	17	3.106	0.125	5.374	0.520
	October	17	2.575	0.195	2.962	0.424
H. radiatus ♀	September	8	6.263	0.482	8.910	1.52
	October	11	4.823	0.369	8.159	0.898

Fig. 5.1a-g. The change in thorax and abdomen masses of adult males from nine species of Limnenphilid caddis flies over the course of their flight periods. Data are pooled into groups on the basis of capture date, and are presented as a percentage of the mean mass of the first sample group. The standard errors have been omitted from these figures for clarity, and the sample sizes and actual mean values (with standard errors) for each sample group are displayed in Table 5.1. Statistics are from regressions of either log thorax mass or log abdomen mass against capture date, for ungrouped data.



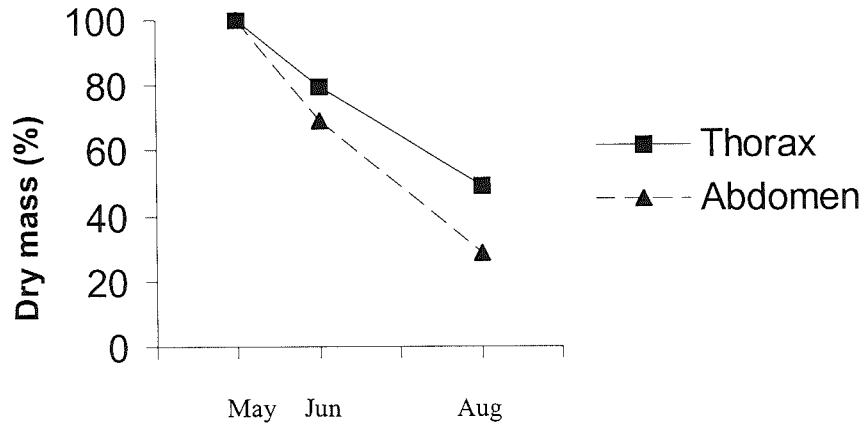
(a) *Apatania wallengreni*

Thorax: $r^2_{(adj)} = 0.0\%$, $p = 0.421$, df = 19; Abdomen: $r^2_{(adj)} = 31.8\%$, $p = 0.006$, df = 19



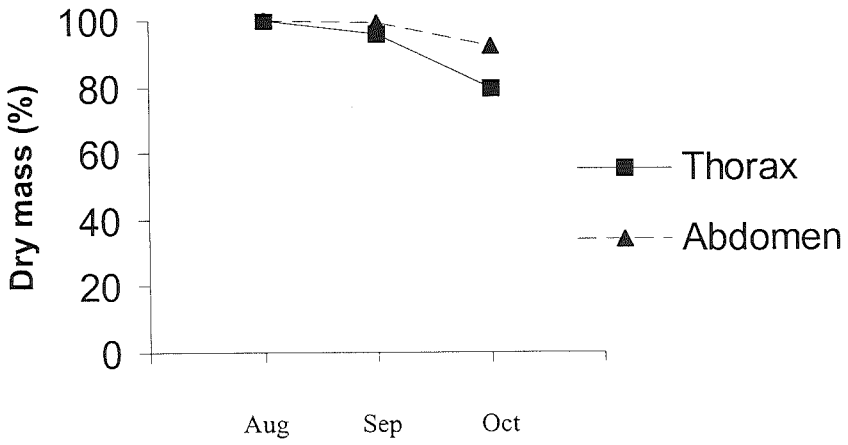
(b) *Glyphotaelius pellucidus*

Thorax: $r^2_{(adj)} = 47.0\%$, $p < 0.001$, df = 32; Abdomen: $r^2_{(adj)} = 59.0\%$, $p < 0.001$, df = 32



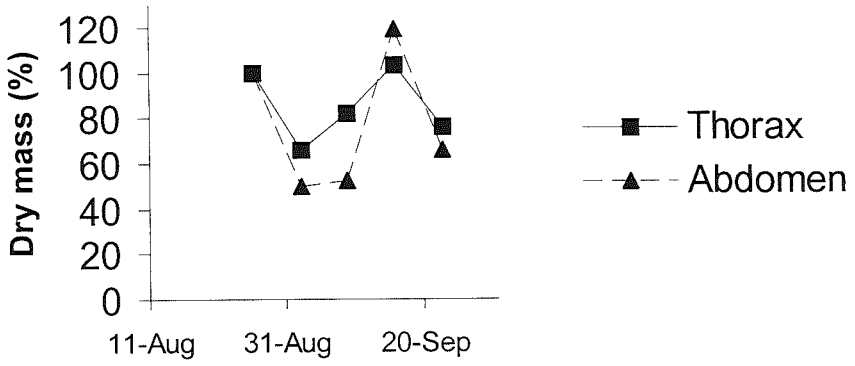
(c) *Micropterna lateralis*

Thorax: $r^2_{(adj)}$ = 48.0%, p = 0.007, df = 11; Abdomen: $r^2_{(adj)}$ = 48.3%, p = 0.007, df = 11



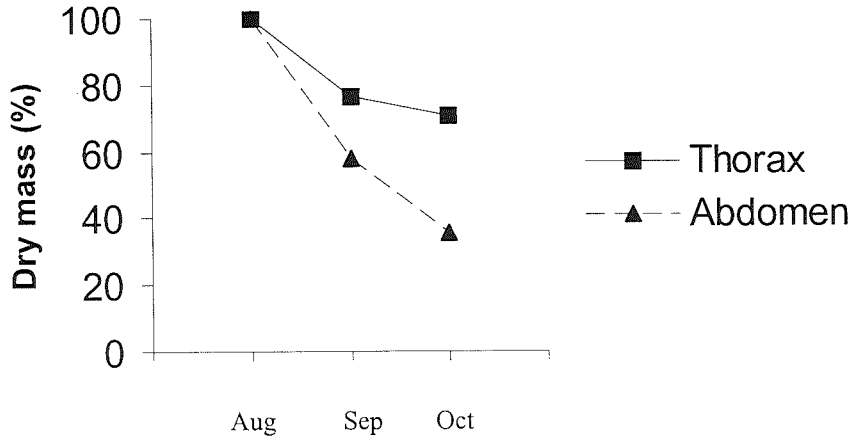
(d) *Limnephilus marmoratus*

Thorax: $r^2_{(adj)}$ = 0.2%, p = 0.316, df = 23; Abdomen: $r^2_{(adj)}$ = 0.0%, p = 0.755, df = 23



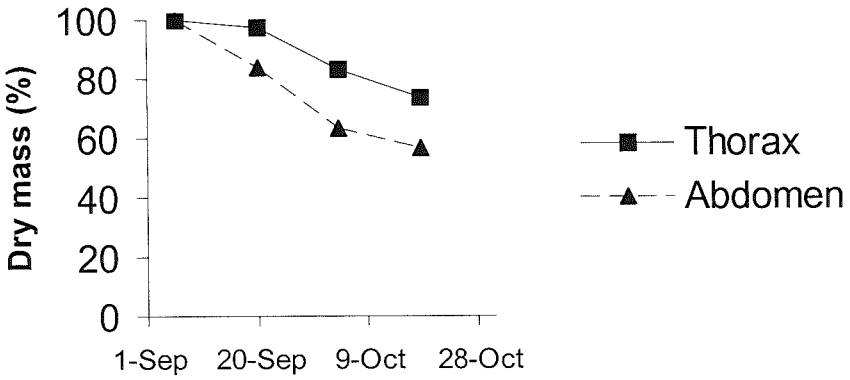
(e) *Limnephilus sparsus*

Thorax: $r^2_{(adj)} = 0.0\%$, $p = 0.562$, df = 24; Abdomen: $r^2_{(adj)} = 0.0\%$, $p = 0.832$, df = 24



(f) *Limnephilus lunatus*

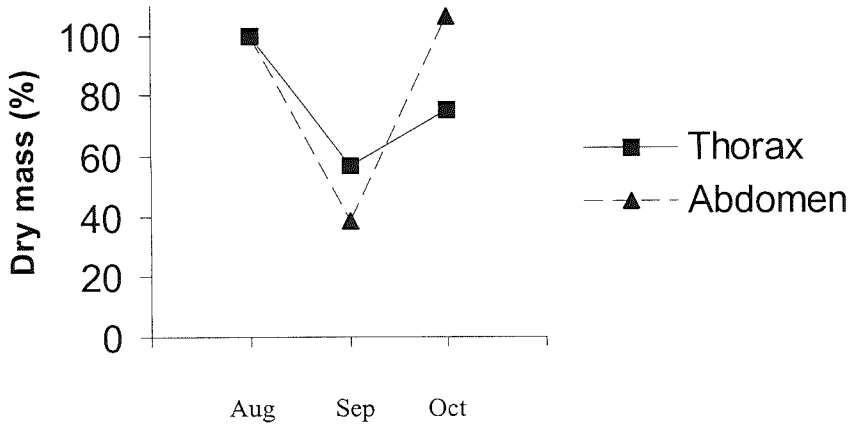
Thorax: $r^2_{(adj)} = 23.1\%$, $p < 0.001$, df = 93; Abdomen: $r^2_{(adj)} = 36.2\%$, $p < 0.001$, df = 93



(g) *Halesus radiatus*

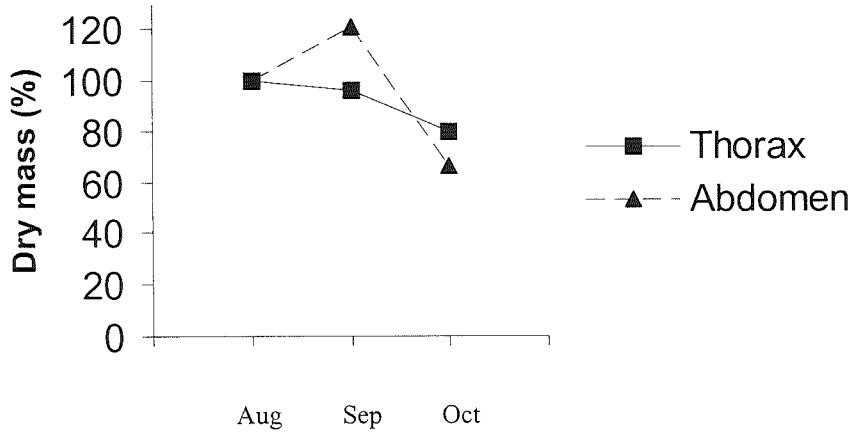
Thorax: $r^2_{(adj)} = 3.1\%$, $p = 0.098$, df = 57; Abdomen: $r^2_{(adj)} = 7.0\%$, $p = 0.025$, df = 57

Fig. 5.2a-c. The change in thorax and abdomen masses of adult females from three species of Limnenphilid caddis flies over the course of their flight periods. Data are pooled into groups on the basis of capture date, and are presented as a percentage of the mean mass of the first sample group. The standard errors have been omitted from these figures for clarity, and the sample sizes and actual mean values (with standard errors) for each sample group are displayed in Table 5.1. Statistics are from regressions of either log thorax mass or log abdomen mass against capture date, for ungrouped data.



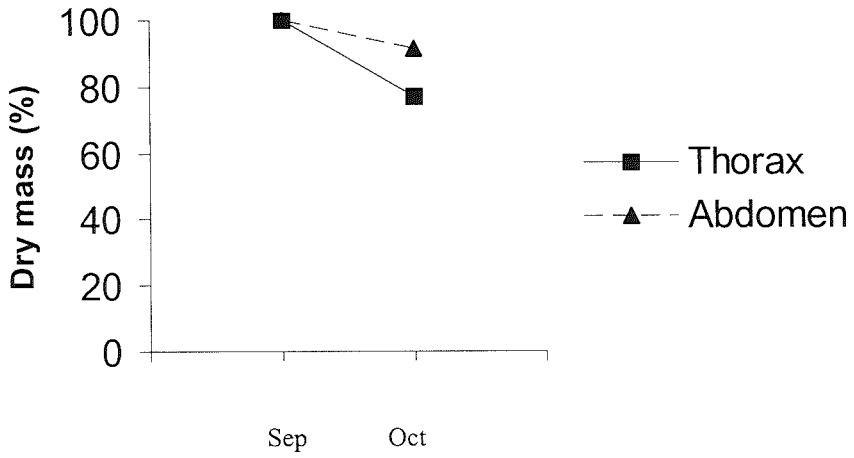
(a) *Limnephilus marmoratus*

Thorax: $r^2_{(adj)} = 43.1\%$, $p = 0.033$, df = 8; Abdomen: $r^2_{(adj)} = 0.0\%$, $p = 0.410$, df = 8



(b) *Limnephilus lunatus*

Thorax: $r^2_{(adj)} = 5.5\%$, $p = 0.079$, df = 39; Abdomen: $r^2_{(adj)} = 8.1\%$, $p = 0.042$, df = 39



(c) *Halesus radiatus*

Thorax: $r^2_{(adj)} = 33.4\%$, $p = 0.006$, df = 18; Abdomen: $r^2_{(adj)} = 0.0\%$, $p = 0.522$, df = 18

Table 5.2. The change in flight ability of adult Limnephilid caddis flies during their lifetimes. Wing length is in mm and wing loading is calculated as (dry thorax mass (mg) + dry abdomen mass (mg))/ wing length^2). TAR is the ratio of dry thorax mass to dry abdomen mass. Data was logged and regressed against capture date.

Species			df	$r^2_{(adj)}$ %	*p*	
Apatania wallengreni	♂	wing	18	0.0	0.771	
		wing loading	18	11.2	0.088	
		TAR	19	22.1	0.021	*
Glyphotaelius pellucidus	♂	wing	32	5.1	0.109	
		wing loading	32	62.4	<0.001	*
		TAR	32	33.3	<0.001	*
Micropterna lateralis	♂	wing	11	14.1	0.125	
		wing loading	11	44.6	0.011	*
		TAR	11	25.2	0.055	*
Limnephilus marmoratus	♂	wing	23	0.0	0.842	
		wing loading	23	0.0	0.753	
		TAR	23	0.7	0.294	
Limnephilus marmoratus	♀	wing	8	16.1	0.155	
		wing loading	8	0.0	0.388	
		TAR	8	0.0	0.585	
Limnephilus sparsus	♂	wing	24	0.0	0.474	
		wing loading	24	0.0	0.877	
		TAR	24	0.0	0.392	
Limnephilus lunatus	♂	wing	92	0.4	0.246	
		wing loading	92	44.8	<0.001	*
		TAR	93	27.1	<0.001	*
Limnephilus lunatus	♀	wing	39	4.3	0.105	
		wing loading	39	19.2	0.003	*
		TAR	39	0.0	0.347	
Halesus radiatus	♂	wing	54	0.0	0.546	
		wing loading	54	7.9	0.021	
		TAR	56	5.1	0.049	
Halesus radiatus	♀	wing	18	9.8	0.104	
		wing loading	18	0.0	0.619	
		TAR	18	0.0	0.348	

* Significant relationships after application of the sequential Bonferroni technique (see Methods for details)

5.3.2. Fat & muscle digestion.

The removal of protein from thoraces and fat from abdomens of a sub-sample of the light trapped individuals gave conflicting results (Table 5.3.). The decline in thorax mass was accompanied by a change in exoskeleton mass for male *G. pellucidus*, however no change in exoskeleton was recorded for male *L. lunatus* or females of *L. marmoratus* and *H. radiatus*, despite a decrease in overall thorax mass. In all cases where abdomen mass declined, so did lean abdomen mass, except for males of *L. lunatus*.

5.3.3. Mass and nitrogen content changes in captive reared adults.

Four species had sample sizes sufficient to examine the change in mass and nitrogen over the course of the life span (Table 5.4). The thorax mass of *G. pellucidus* decreased over the life span for both sexes. There was no change in abdominal mass, but the sexes did differ in abdomen size. Results for *G. pellucidus* are different to those found in light trapped individuals where both thorax and abdomen mass declined over time (i.e. life span) (see Table 5.1.). The amount of nitrogen (mg) in the thorax followed a similar pattern to mass, and decreased. Also in line with mass changes, abdomen nitrogen showed no significant increase. However, analysis of just females indicates they may be increasing their abdomen nitrogen content with age (GLM: Life span $F_{1,14} = 4.64, p = 0.051$).

Abdominal mass and nitrogen content significantly increased for both sexes in *L. rhombicus,* whereas thorax mass showed no significant change. Although thorax mass did not change for *L. rhombicus*, thorax nitrogen content in fact decreased in both males and females. *P. cingulatus* showed no mass changes in either thorax or abdomen. Despite this, *P. cingulatus* adults of both sexes lost nitrogen from both the thorax, and also strangely, the abdomen. As such it was the only species to show a significant decrease in total nitrogen content (GLM on total nitrogen with life span as covariate: Sex $F_{1,12} = 8.64, p = 0.015$, Life span $F_{1,12} = 7.49, p = 0.021$). No *O. albicorne* individuals showed any change in mass or nitrogen content over the course of their life spans, from thoraces or abdomens.

Table 5.3. The change in log thorax mass, log thorax-exoskeleton mass, log abdomen mass and log lean-abdomen mass over the flight period for six of the Limnephilid species caught in the light trap. Statistics are from regressions against capture date.

Species			df	r^2 %$_{(adj)}$	*p*	
Apatania wallengreni	♂	thorax	19	0.0	0.421	
		abdomen	19	31.8	0.006	*
		lean abdomen	12	57.7	0.002	*
Glyphotaelius pellucidus	♂	thorax	32	47.0	<0.001	*
		thorax (- muscle)	9	45.9	0.019	*
		abdomen	32	59.0	<0.001	*
		lean abdomen	6	67.8	<0.001	*
Limnephilus marmoratus	♂	thorax	23	0.2	0.316	
		thorax (- muscle)	7	0.0	0.407	
		abdomen	23	0.0	0.755	
		lean abdomen	8	0.0	0.785	
Limnephilus marmoratus	♀	thorax	8	43.1	0.033	*
		thorax (- muscle)	7	2.3	0.321	
		abdomen	8	0.0	0.410	
		lean abdomen	6	0.0	0.765	
Limnephilus sparsus	♂	thorax	24	0.0	0.562	
		thorax (-muscle)	8	17.8	0.142	
		abdomen	24	0.0	0.832	
		lean abdomen	8	22.7	0.110	
Limnephilus lunatus	♂	thorax	93	23.1	<0.001	*
		thorax (- muscle)	19	0.0	0.670	
		abdomen	93	36.2	<0.001	*
		lean abdomen	19	11.3	0.101	
Limnephilus lunatus	♀	thorax	39	5.5	0.079	
		thorax (- muscle)	11	19.5	0.085	
		abdomen	39	8.1	0.042	
		lean abdomen	11	0.0	0.615	
Halesus radiatus	♂	thorax	57	3.1	0.098	
		thorax (-muscle)	8	0.0	0.987	
		abdomen	57	7.0	0.025	
		lean abdomen	8	79.9	<0.001	*
Halesus radiatus	♀	thorax	18	33.4	0.006	*
		thorax (- muscle)	6	52.7	0.039	
		abdomen	18	0.0	0.522	
		lean abdomen	6	1.1	0.349	

* Significant relationships after application of the sequential Bonferroni technique (see Methods for details)

Table 5.4. The change in thoracic (Th) and abdominal (Ab) mass and nitrogen content during the lifetime of individuals from four species of caddis flies reared in captivity. Results are from general linear models (GLM) using sex as a factor and life span as a covariate.

Species		Sex	*p*	Life span	*p*
Glyphotaelius pellucidus	Th mass	$F_{(1,23)} = 0.18$	0.680	$F_{(1,23)} = 7.88$	0.011 *
	Th nitrogen	$F_{(1,23)} = 0.25$	0.620	$F_{(1,23)} = 12.77$	0.002 *
	Ab mass	$F_{(1,23)} = 11.32$	0.003 *	$F_{(1,23)} = 0.06$	0.803
	Ab nitrogen	$F_{(1,23)} = 3.63$	0.070	$F_{(1,23)} = 2.03$	0.169
Limnephilus rhombicus	Th mass	$F_{(1,13)} = 2.39$	0.150	$F_{(1,13)} = 1.22$	0.293
	Th nitrogen	$F_{(1,13)} = 0.01$	0.920	$F_{(1,13)} = 8.00$	0.016 *
	Ab mass	$F_{(1,13)} = 1.01$	0.335	$F_{(1,13)} = 13.19$	0.004 *
	Ab nitrogen	$F_{(1,13)} = 26.43$	<0.001*	$F_{(1,13)} = 8.90$	0.012 *
Potomophylax cingulatus	Th mass	$F_{(1,12)} = 0.03$	0.866	$F_{(1,12)} = 0.13$	0.722
	Th nitrogen	$F_{(1,12)} = 14.87$	0.003 *	$F_{(1,12)} = 6.68$	0.027 *
	Ab mass	$F_{(1,12)} = 5.72$	0.038 *	$F_{(1,12)} = 0.00$	0.999
	Ab nitrogen	$F_{(1,12)} = 4.19$	0.068	$F_{(1,12)} = 4.93$	0.051 *
Odontocerum albicorne	Th mass	$F_{(1,14)} = 4.13$	0.065	$F_{(1,14)} = 0.00$	0.958
	Th nitrogen	$F_{(1,14)} = 3.73$	0.077	$F_{(1,14)} = 0.03$	0.867
	Ab mass	$F_{(1,14)} = 114.7$	<0.001*	$F_{(1,14)} = 1.41$	0.259
	Ab nitrogen	$F_{(1,14)} = 68.63$	<0.001*	$F_{(1,14)} = 3.59$	0.085

* Significant relationships after application of the sequential Bonferroni technique (see Methods for details)

5.4. DISCUSSION

The loss of body mass from either the thorax or abdomen occurred in a number of the species collected from the light trap. Mass changes are all attributable to losses during the lifetime of individuals and not a seasonally related decline in overall size. None of these species displayed a decline in wing length during the flight period, which would be indicative of long emergence periods. Therefore all have short emergence periods relative to their flight periods. Such resource dynamics can be viewed in terms of reproductive investment, and are known to be associated with mating system in butterflies (Karlsson 1987, 1994, 1998, Stejrnholm & Karlsson 2000). Unlike butterflies, the mating systems of caddis flies are poorly known (Hoffmann 1999, Arnqvist *et al.* 2000), and so these results could be used to demonstrate the relative investment made by the sexes, which in turn could be used to predict the degree of polyandry within species (see Chapter 7).

Males of three species caught in the light trap, *G. pellucidus*, *M. lateralis* and *L. lunatus*, displayed a decline in thoracic as well as abdominal mass. In butterflies, such a pattern is associated with polyandrous mating systems (Karlsson 1998, Stejrnholm & Karlsson 2000), where males appear to break down flight muscle to provide nitrogen for reproduction. In captivity, *G. pellucidus* again demonstrated declining thoracic mass (and thorax nitrogen content). Males from a fourth species *L. rhombicus*, did not display a loss of thorax mass but did show a decrease in its nitrogen content, and they increased the nitrogen content of their abdomens when reared in captivity. With no other source of nitrogen, this abdominal increase could only occur through incorporating nitrogen derived from the thorax into the abdomen. On the basis of what is known from Lepidopterans, these four species can therefore be predcited to be polyandrous. Four other species from the light trap (*A. wallengreni*, *H. radiatus*, *L. marmoratus* and *L sparsus*) did not demonstrate such a decline in thorax mass. In monandrous butterfly species, thoracic mass loss does not occur in males (Karlsson 1987, 1994), so these caddis fly species can be predicted to be monandrous. Of the other species in captivity, *O. albicorne* also appeared to be monandrous from its resource dynamics (as was previously suggested in Chapter 2). *P. cingulatus* did not display any mass changes, but did display a decrease in nitrogen from both thorax

and abdomen, and therefore in total nitrogen level. The significance of this is unclear and requires further investigation.

The pattern for female caddis flies was slightly different to that of butterflies. In butterflies, females appear to breakdown flight muscle regardless of the mating system (i.e. monandry or polyandry), and the use of thorax-derived resources in reproduction possibly even increases with repeated matings (Karlsson 1998). In contrast, in individuals from the light trap, loss of thoracic mass occurred in the females of species where the pattern of resource use in the male indicated monandry (*L. marmoratus*, *H.radiatus*). However, females of *L. lunatus* (where male resource use indicates polyandry) did not display a loss of thorax mass. It is possible that the decline in thorax mass only occurs late in the life span for these females, and such individuals were not collected because sampling finished prior to the end of the flight period. In captivity, *G. pellucidus* females were consistent with the pattern from butterflies, and *L. rhombicus* females also showed this pattern, if only for nitrogen and not for mass. As with the males, female *O. albicorne* did not display mass losses, but flight muscle breakdown will most likely only occur in species that continue to produce eggs after eclosion. *O. albicorne* emerges with its lifetime supply of eggs already developed and so there is no requirement for nitrogen from the thorax to be used for reproduction.

It is possible there are other explanations for these patterns of resource dynamics. Across species, mean male thoracic mass loss was positively correlated with long flight periods (and hence long life spans) ($r^2_{(adj)} = 69.1\%$, $p = 0.007$, $df = 7$). However, there was no such significant relationship between abdomen mass and flight period ($r^2_{(adj)} = 26.6\%$, $p = 0.135$, $df = 6$). This may indicate that the nature of the thoracic mass declines are related to somatic use, and is therefore positively associated with length of life. However, if abdomen mass loss is mainly reproductive in nature (i.e. ejaculate expenditure), it may have no association with life span. Equally, the decline in abdomen mass may also be linked to other processes that are not directly reproductive (i.e. flight energetics). The loss of thorax mass may be related to somatic functions. The ability to repair 'wear and tear' may be restricted in adults with limited feeding (Boggs 1992), and thorax mass has been found to be related to longevity in Lepdiopterans (Karlsson & Wickman 1989, Gage 1995). Flight muscle may therefore

just degenerate over time (Marden 2000), as the synthesis of new proteins will most likely be limited in organisms with restricted nitrogen intake (Boggs 1992).

Data from the protein digestions suggests that a decline in tissues other than muscle may be responsible for the mass loss from the thoraces of some species. The thin layer of fat in the thorax, or even cuticular compounds could decline over the lifespan (J. Marden *pers. comm*) to produce the observed pattern. However, if thoracic mass loss was simply a function of longevity, it would be expected to decrease in a similar manner for males and females of the same species, which is not the case. Also, in at least one species, nitrogen from the thorax appears to have been incorporated into the abdomen. It is difficult to prove that abdominal losses were purely reproductive in nature, but the fat digestions did indicate that lean abdomen masses decreased in a similar manner to whole abdomens i.e. that nutrients other than lipids were being expended. For males at least this would tend to imply the mass loss was due to reproductive output as spermatophores are low in lipids (Marshall 1982). Comparisons of the resource budget between wild and captive individuals are somewhat confounded because captive individuals will have been less active, have had a greater access to food and were denied mating opportunities. This may explain why nitrogen and mass changed at different rates in some captive individuals. Despite this, changes in nitrogen levels of individuals held singly in captivity also demonstrate that the variations in mass observed in the wild are not solely a result of energetic expenditure, but are related to the use of body resources for reproduction.

The changes in the proportion of body mass allocated to thorax and abdomen will have obvious consequences for flight (Srygley 1994). In all cases where either TAR changed, it did so in a manner that is indicative of improved flight ability (Chai 1996). High ratios of thorax (flight muscle) mass to abdomen (body) mass are positively associated with flight speeds (Srygley & Dudley 1993) and flight frequencies (Kingsolver & Srygley 2000) in butterflies. This could be important in mate finding and predator avoidance, as has been shown for lepidopteran adults (Srygley & Kingsolver 1998). Wing loading, which is also positively correlated with flight speeds (Srygley & Dudley 1993), is not such a good measure of locomotory ability in these circumstances because the relative proportions of flight muscle to the rest of the body are changing. This means a decrease in wing loading will actually be

associated with an improved flight ability (Kingsolver & Srygley 2000). To what extent flight ability plays a part in the ecology of adult caddis flies has previously only been determined for smaller, swarming species such as many Leptocerids (Petersson 1989, 1995). Improving flight ability or flight frequency over the course of the life span may explain why in some species greater numbers are caught in light traps late on in the season, compared to the beginning of the flight period (Chapter 4).

Resource allocation in caddis flies is interesting because adults rely heavily on larval derived nutrients, and in such species the amount of these accrued will limit an individual's fitness (Karlsson 1995). Not only that, but allocation decisions during metamorphosis may also constrain life history traits (Chapter 2 & 3). However, resource allocation may show great flexibility over varying timescales, which allows adult caddis flies to achieve their behavioural goals whilst coping with the environmental conditions they encounter during their life span (Piersma & Lindström 1997). This cross species comparison of adults shows that caddis flies, like butterflies, have diverse resource allocation strategies. In the wild, males of a number of species move resources from thoracic to abdominal regions. Based on data from butterflies, this suggests that these species should be polyandrous. No change in male resource allocation was found for a number of other species, suggesting that these species are monandrous. Resource dynamics in female caddis flies also generally agreed with patterns from butterflies. Changes in adult resource allocation were also observed in captive individuals, indicating they are not just a consequence of energy expenditure on flight or lack of foraging opportunities, but as in butterflies, linked to the use of body resources in reproduction. More information is needed however on exactly which resources are involved, and what situations bring about a re-allocation, as well as how and why sexes and species differ in their patterns of resource use.

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Chapter 6. Seasonal changes in adult body size and resource allocation.

6.1. INTRODUCTION

Body size plays a central part in current life history theories (Peters 1983, Reiss 1989, Roff 1992, Stearns 1992), and it has particularly important implications for the fitness of adult insects (Choe & Crespi 1997). As well as the decline in body mass that occurs during the life of non-feeding adult stages (Chapter 5), structural body size at emergence can also vary due to a range of conditions operating within the juvenile stages. Generally, it has been considered that there is a positive relationship between age and size at maturity (Stearns 1992), but recent empirical work with arthropods has in fact shown a negative correlation (e.g. Nylin *et al.*1996, Higgins 2000). In insects with long emergence periods, juveniles will experience a range of environmental conditions as the season progresses. These environmental conditions during the juvenile stages often play a major part in determining insect body size (Sebens 1987) and as a consequence there can be significant size variation in emerging adults over the course of a season. For instance, increasing temperature and declining quality of the environment leads to individuals that whilst maturing at the same age, become progressively smaller as the season advances. This has been observed in a range of univoltine arthropod taxa (Atkinson 1994, Higgins & Rankin 1996, but see Klingenberg & Spence 1997).

Whilst large body size in insects has generally been postulated to be an advantage (Honek 1993) there is a growing amount of evidence that body size has no effect or even negative effects on fitness (Blackenhorn in press, Sokolovska *et al.* 2000). One reason for this may be that it is not body size *per se* ('the amount of resources'), but the way organisms allocate these resources to different structures or functions that is the most crucial aspect for optimising their fitness (Angelo & Slansky 1984). Smaller individuals obviously have less absolute resources to allocate and if total resource levels are lower, then the manner in which they are invested may vary (i.e. an allometric effect) (Dixon & Kindlmann 1999). Very little information is available as to whether allocation patterns stay constant, despite changes in body sizes over a season, or whether early and late eclosing individuals pursue different strategies. Dixon & Kundu (1998) found that early season generations of the aphid *Cavariella aegopodii* had larger gonads and less lipid reserves than later generations,

regardless of body size, and that this was a response to changing food quality. Female amphipods produce larger offspring in winter (Glazier 2000). There is also some evidence from studies on butterflies that different seasonal forms vary in their allocation to soma or reproduction (Angelo & Slansky 1984, Bradby & Jones 1995).

The decrease in size of newly eclosed adults over time, in species with extended emergence periods, is also seen in caddis flies (see Hayashi 1990). Again, food quality and environmental temperature in the larval habitat have been suggested as the causative factors of this pattern (Svensson 1975, Petersson 1989). In a bivoltine *Stenopsyche marmorata* population in Japan, body size of newly emerged adults decreased over the year, which was assumed by the authors to be a temperature mediated effect. Gamete size in these newly eclosed individuals however initially decreased from spring to summer, but then increased from summer to autumn (Hayashi *et al.*1999). This implies that body and gamete sizes are affected independently by the environment, or this may be a result of differential allocation strategies (see Avelar 1993, Wiklund *et. al.*1987).

Therefore, it is possible that individuals growing late in the season allocate their resources differently, either as a direct consequence of changing environmental conditions on development, or because the requirements and selection pressures of the adult stage vary with body size (Blackenhorn *in press*). To determine whether body size and resource allocation patterns do change within a season, adults from two short-lived species that have extended emergence periods, *Lepidostoma hirtum* and *Plectronemia conspersa* (Svensson 1972, Crichton & Fisher 1978) were collected using a light trap over the course of their flight periods. The exact nature of their life cycles are not well known, except that there are 5th instar larvae and pupae present during all of spring and summer (M. Hansell *pers.com.*, Sangpradub *et al.* 1999) and that more than one generation per year is possible (M. Hansell *pers.com.*, Edington & Hildrew 1995). Whatever the exact life cycle, there will be same-age larvae developing and adults emerging right through the period studied. Structural body size (wing length) and resource allocation (thorax & abdomen mass, flight muscle and fat contents) were measured. Changes in the ratio of mass to wing size (wing loading) or thorax to abdomen will have obvious effects on flight ability (Srygley & Dudley 1993), and changes in abdomen mass will have obvious consequences for reproduction (Boggs 1981, Karlsson 1987).

6.2. METHODS

Male and female *Lepidostoma hirtum* and *Plectronemia conspersa* used for body size and mass analysis were obtained from light trap samples in 1997. The number of individuals caught in 1998 and 1999 was also recorded, although no size or mass measurements were taken from these individuals. Wet mass was measured to the nearest 0.01mg and right fore-wing length to the nearest 0.1mm using a dissecting microscope with an occular micrometer. Wings, legs and the head were then removed, and the remaining thorax-abdomen dried for three days in an oven at 60°C. These were then separated, weighed to the nearest 1μg, and then frozen. Once all individuals were processed in this way, a number were selected for flight muscle and fat content analysis.

As in Chapter 5, dried thoraces and abdomens from individuals selected for analysis were re-dried at 60° c for 24 hours. Individual body parts were weighed and then each placed in a 0.5ml eppindorf tube. Each tube was filled with either 30% Potassium Hydroxide solution (to dissolve the muscle from thoraces) or Diethyl Ether (to dissolve the fat from abdomens), and left for 24 hours. They were then centrifuged for 10 minutes at 13,000 rpm, and the supernatant decanted. The tubes were then filled for the second time with the appropriate reagent, left for 24 hours and centrifuged as before. After removing the supernatant, distilled water was added, and the tubes centrifuged again. For the thoraces this was repeated twice more. Thoraces and abdomens were again dried for 24 hours at 60° c and re-weighed.

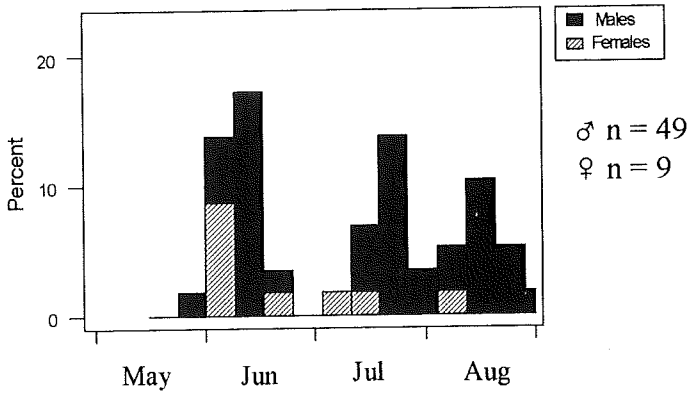
All body size, body mass and resource allocation data were log transformed prior to regression against capture date (Julian Days). Data for males and females were analysed separately. To adjust for the number of tests performed, the sequential Bonferroni technique (Holm 1979) was applied to statistical tests performed within each sex of each species. As suggested by Wright (1992) and Chandler (1995), the critical value was set to 10%.

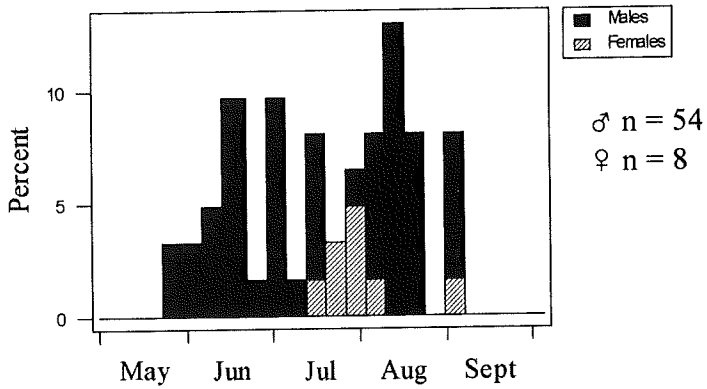
6.3. RESULTS

Fig. 6.1 shows the flight periods for the two species, which are essentially measures of the emergence period because they are both short lived species. Therefore it can be assumed that all conspecific individuals caught in the light trap are roughly the same age, regardless of capture date. All relationships remained significant when the critical value (equivalent to the 10% confidence level; Wright 1992, Chandler 1995) was adjusted to allow for the fact that multiple tests were performed. A significant decrease in structural body size (i.e. wing length) over the flight period was observed in both sexes for *Lepidostoma hirtum* (Table 6.1 & Fig 6.2a : males: $r^2_{(adj)} = 39.8\%$, $p < 0.001$, $df = 48$; Fig. 6.2b females: $r^2_{(adj)} = 74.2\%$, $p < 0.001$, $df = 8$). For *Plectronemia conspersa* there were only enough data for males and the trend for decreasing size at emergence was not significant (Table 6.1 & Fig. 6.2c: males: $r^2_{(adj)} = 16.2\%$, $p = 0.107$, $df = 11$).

As well as displaying a seasonal decrease in structural body size as measured by wing length, *L. hirtum* males showed a decrease in thorax (Table 6.1: $r^2_{(adj)} = 46.6\%$, $p < 0.001$, $df = 47$) and abdomen (Table 6.1: $r^2_{(adj)} = 21.7\%$, $p < 0.001$, $df = 47$) mass. Despite the mass changes, *L. hirtum* maintained a constant Thorax : abdomen ratio (TAR) (Table 6.2.: $r^2_{(adj)} = 0.0\%$, $p = 0.808$, $df = 47$). Wing loading however decreased (Table 6.2.: $r^2_{(adj)} = 48.9\%$, $p < 0.001$, $df = 47$). *P. conspersa* on the other hand showed no significant mass changes during the season in either thorax (Table 6.1.: $r^2_{(adj)} = 0.0\%$, $p = 0.546$, $df = 12$) or abdomen (Table 6.1.: $r^2_{(adj)} = 0{,}0\%$, $p = 0.427$, $df = 12$). As such its TAR was also unaffected (Table 6.2.: $r^2_{(adj)} = 0.0\%$, $p = 0.509$, $df = 12$) as was its wing loading (Table 6.2: $r^2_{(adj)} = 0.0\%$, $p = 0.629$, $df = 11$).

Due to a laboratory accident, the thorax samples for muscle-analysis from *L. hirtum* males were destroyed. Despite the decrease in male abdomen mass as the season progressed, the fat content of male *L. hirtum* abdomens increased proportionally (Table 6.2 & Fig. 6.3a: $r^2_{(adj)} = 33.2\%$, $p = 0.007$, $df = 17$) but not absolutely ($r^2_{(adj)} = 5.9\%$, $p = 0.171$, $df = 17$). Data for female fat content was only collected from three individuals, but this seemed to increase as well (Fig. 6.2b). Neither muscle ($r^2_{(adj)} = 0.0\%$, $p = 0.397$, $df = 8$) nor fat content (Table 6.2 & Fig. 6.2c: $r^2_{(adj)} = 0.0\%$, $p = 0.399$, $df = 8$) changed seasonally for *P. conspersa* males.





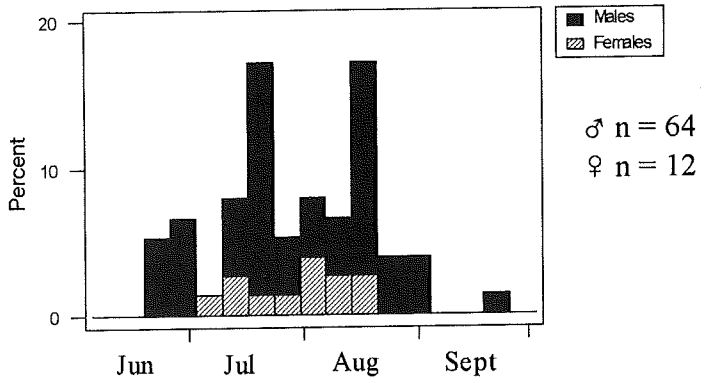
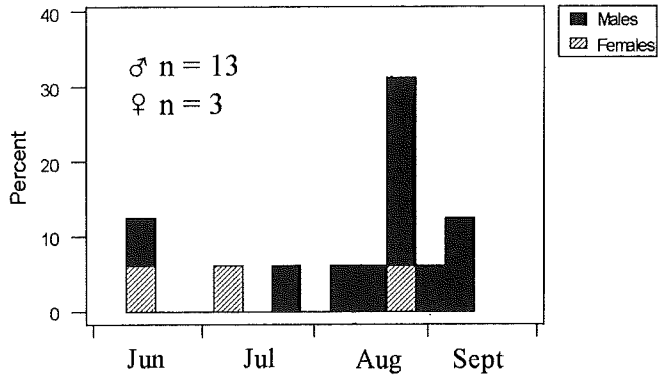
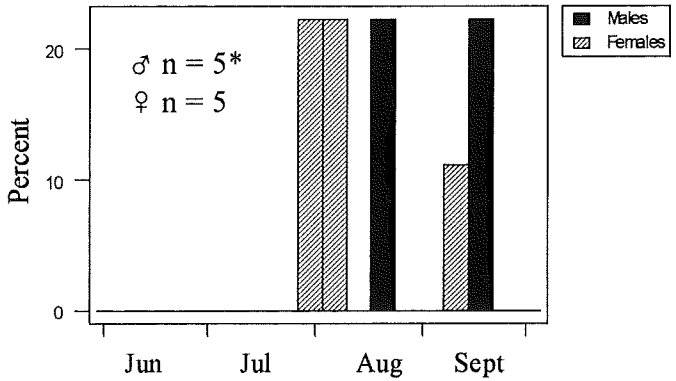


Fig6.1a. Flight periods of *Lepidostoma hirtum* caught in a light trap, 1997-1999. Data are presented as weekly totals expressed as a percentage of the total yearly capture.





*plus one male caught on the 29/5/99

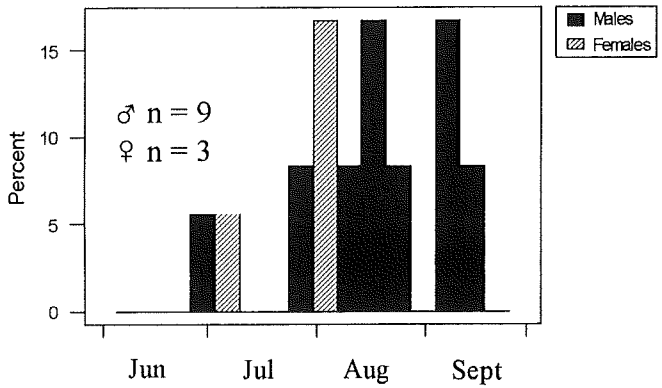


Fig.6.1b Flight periods of *Plectronemia conspersa* caught in a light trap, 1997-1999. Data are presented as weekly totals expressed as a percentage of the total yearly capture.

Table 6.1 The mean monthly wing length (mm) and dry-mass (mg) of adult thoraces and abdomens of males from two caddis fly species.

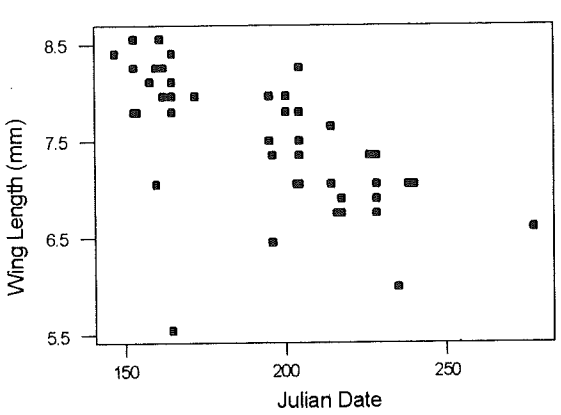
		n	Wing	se	Thorax	se	Abdomen	se
L. hirtum	May	1	8.40	-	0.694	-	0.575	-
	June	20	7.98	0.15	0.567	0.027	0.328	0.033
	July	14	7.48	0.13	0.438	0.024	0.227	0.031
	August	12*	6.91	0.09	0.326	0.035	0.177	0.026
	October	1	6.60	-	0.217	-	0.209	-
P. conspersa	May	2	10.20	0.15	1.613	0.229	1.069	0.199
	June	1	9.90	-	1.450	-	0.493	-
	July	7	9.77	0.14	1.469	0.087	0.716	0.112
	August	3**	9.60	0.30	1.484	0.201	0.693	0.114

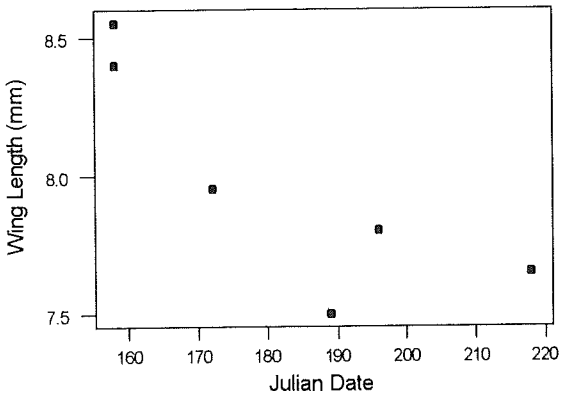
* sample size for wings n=13 ** sample size for wings n= 2

Table 6.2 The mean monthly thorax: abdomen ration (TAR), wing loading (mg/mm^2) and abdomen fat content (%) of males from two caddis fly species.

		n	TAR	se	Wing loading	se	*n*	Fat content	se
L. hirtum	May	1	1.21	-	1.27	-	1	8.09	-
	June	20	2.05	0.19	0.89	0.05	10	7.22	1.79
	July	14	2.28	0.24	0.67	0.04	3	6.9	1.59
	August	12	2.22	0.31	0.50	0.05	4	38.7	10.70
	October	1	1.04	-	0.43	-	-	-	-
P. conspersa	May	2	1.52	0.07	2.68	0.43	1	5.72	-
	June	1	2.94		1.94	-	1	2.77	-
	July	7	2.42	0.42	2.19	0.17	5	54.5	13.30
	August	2*	2.17	0.16	2.48	0.02	2	9.29	1.45

* sample size for TAR n = 3





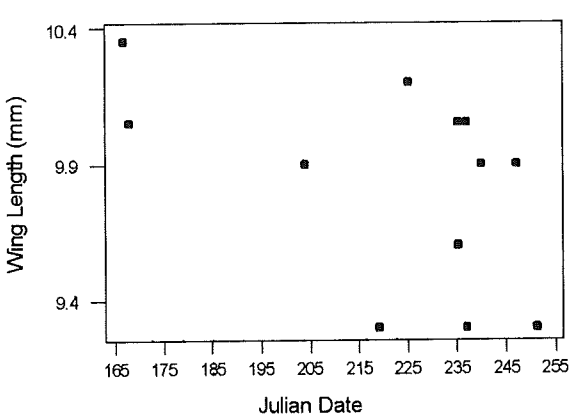
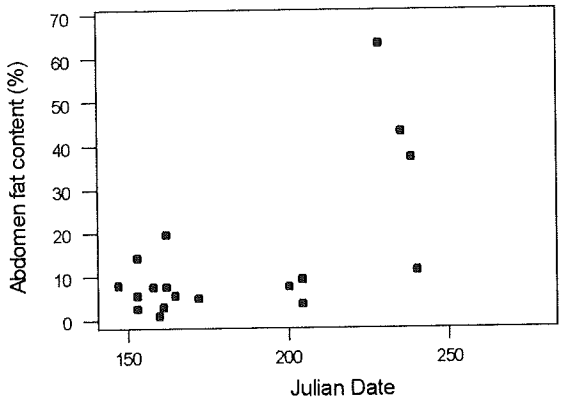
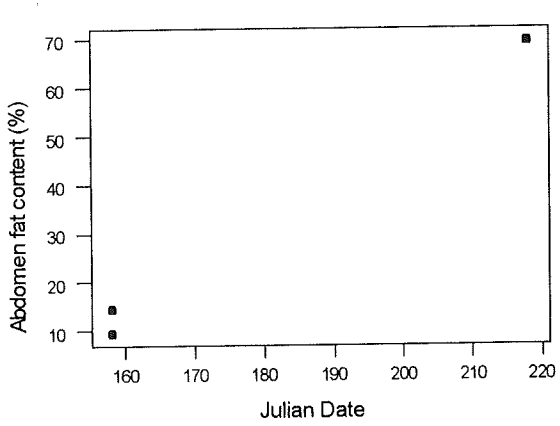


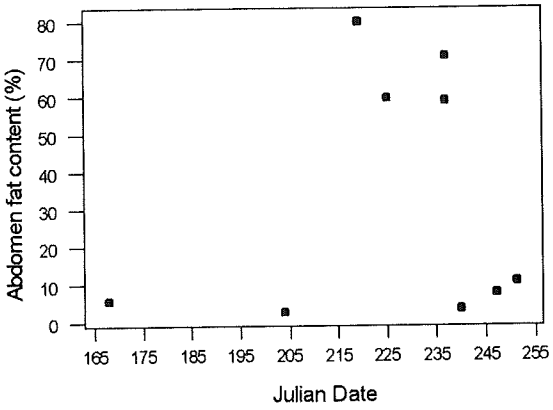
Fig. 6.2. The relationship between adult wing length and capture date for (a) male and (b) female *Lepidostoma hirtum* and (c) male *Plectronemia conspersa* caught in a light trap.



(a)



(b)



(c)

Fig. 6.3. The relationship between abdomen fat content (%) and capture date for (a) male and (b) female *Lepidostoma hirtum* and (c) male *Plectronemia conspersa* caught in a light trap.

6.4. DISCUSSION

L. hirtum displayed a very obvious decline in size over its relatively long flight period, which is consistent with results from other univoltine arthropods that have long adult emergence periods and short lifespans, including caddis flies (Resh 1976, Petersson 1989). This pattern is created, it has been suggested, by either larval habitat conditions changing over time (e.g. temperature and food quality) or these smaller individuals being of poor quality (Klingenberg & Spence 1997). It is unlikely that all these individuals are from the same cohort (i.e. they did not hatch from eggs at the same time), and so it is more likely that it is a habitat mediated affect – individuals will be experiencing different conditions during the same developmental stage (Petersson 1989).

There is some evidence of this from other *Lepidostoma* species. Growth of *L. quercina* larvae was found to be limited by food quality in late summer (Grafius & Anderson 1979) and the size of emerging *L. roafi* adults was limited by food quantity (Richardson 1991). *L. hirtum* is a detritivore ('shredder'), and individuals from the light trap would have come from the larval population in Loch Lomond. The amount of course particulate organic matter (CPOM) in the larval habitat will be steadily decreasing through the summer, whist temperatures in the shallow loch margin will be increasing (Fig. 6.4). One, or a combination of both of these are probably responsible for the seasonal decline in size (Sweeney & Vannote 1986, Atkinson & Sibly 1997). *P. conspersa* did not show any size or mass changes even though this species probably also has a similarly extended flight period with short lived adults. However, its larvae can live in fast flowing streams, and so may not be subject to the fluctuations in temperature conditions experienced by *L. hirtum*. Even if these individuals did emerge from the loch and experienced a range of temperatures, *P. conspersa* larvae are carnivorous, and so would not have been subject to the same changes in food conditions.

Contrary to the seasonal decline in size, the proportional fat content of *L. hirtum* adults actually increased over the flight period. Nutritional reserves are known to influence the behaviour of flying insects (Warburg & Yuval 1997), and so this may have been an artefact created by sampling with a light trap. Smaller individuals may have a lesser flight ability and as such only those with the greatest

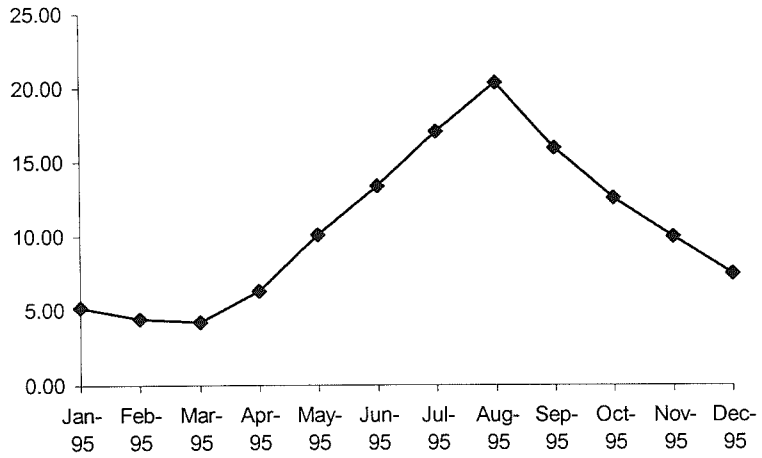


Fig.6.4 Mean monthly water temperatures in the margin of Loch Lomond. Temperatures are in ^{0}C, and were measured during 1995, but the pattern is typical for all years (C. Adams *pers. com.*). Recordings where taken from water where larval *Lepidostoma hirtum* are known to occur, near to the light trap from which the adults used in this study were caught.

energetic reserves would be in flight near the light trap, which is some distance from the shoreline. If this was the case, it could have implications for the interpretation of light trapping studies in general. However, wing loading decreased over the flight period and TAR stayed constant, both of which are consistent with improved flight ability (Kingsolver & Srygley 2000). There was also no obvious decline in numbers as the season progressed in any of the three years, which might be expected if the trap was becoming more 'selective'. It is therefore very possible that late season forms do indeed have different allocation patterns than the larger early season individuals. This may be caused by a switching of diet over the course of the season. Larvae of the shredder *Sericostoma personatum* showed an increase in accumulated fat when fed *Fraxinus* or *Microspora* rather than *Alnus*, despite each diet producing a similar increase in mass (Friberg & Jacobsen 1999). Jacobsen & Sand-Jensen (1994) found that for *Anabolia nervosa* larvae, *Potamegon* and *Alnus* diets cause a difference in the allocation of protein and fat, even though mass gain was again similar. The increasing fat content of *L. hirtum* over time is unlikely to have been caused by any seasonal differences in adult feeding, as nectar does not appear to replenish fat reserves (Petersson & Hasselrot 1994). Larval nutrition has been found to affect adult morphology previously. Angelo & Slansky (1984) found that starved noctuid moth larvae from four different species, produced adults with relatively larger wings and lower wing loadings, and Dixon & Kundu (1998) found a difference in aphid resource allocation due to a seasonally changing quality of diet.

The results from this rather limited study indicate a seasonal difference in resource (fat) allocation in the adults of a species where juveniles of the same developmental stages can experience different habitat conditions, depending on the time of season they are maturing in. Whether the differential allocation occurs purely because of the types of food available in the environment, or through smaller individuals pursuing different resource accumulation or allocation strategies cannot be ascertained from this data, but does warrant further investigation.

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Chapter 7. Resource allocation, sexual size dimorphism and mating systems in caddis flies.

7.1. INTRODUCTION

The behavioural ecology, particularly the reproductive ecology, of adult Trichopterans is poorly known (Hoffmann 1999, Arnqvist 2000). The mating systems of Leptocerids, where adults form swarms prior to pairing, has received some attention in recent years (e.g. Solem 1978, Petersson 1989), and some investigation has been performed into the pheromone production of some Hydropyschids and Rhyacophilids (Phelan 1997 and references therein). However, mating systems within the Limnephilidae have received almost no attention. The Limenphilidae is one of the largest families of caddis flies, and the relatively conspicuous, case-making larvae make up a considerable portion of freshwater benthic communities (Wiggins 1987). The adults also make up a high percentage of the individuals caught in light traps (Crichton 1971,Svensson 1972) so as a group they cannot be considered rare or unimportant. Their general nocturnal habits and long adult life span have however made them a difficult group to study in both the wild or in captivity. In contrast information on the adults of their sister group, the Lepidoptera has not been as difficult to obtain. Their mating systems and morphological correlates are fairly well known, especially within butterflies (Rutowski 1997).

The adult diet of most temperate butterflies is nutrient limited (Baker & Baker 1973, 1986). As such, resources accrued in the larval stage are very important not just for the construction of the adult form, but also for fuelling adult activities such as flight or reproduction (Boggs 1981, Karlsson 1995). These resources are mainly invested into either the thorax or abdomen during metamorphosis, so there is a potential allocation trade-off between them. Function and morphology are closely linked, and so the bodies of such organisms are expected to be adjusted to their life history (Boggs 1981). The resources invested in the thorax at eclosion relative to those in the abdomen are therefore good indicators of several aspects of adult ecology. A large body of work now exists that relates resource allocation patterns in

adult butterflies to several aspects of ecology, including flight ability (Dudley & Srygley 1994), palatability (Marden & Chai 1991), feeding behaviour (Hall & Wilmott 2000), longevity (Karlsson & Wickman 1989), and particularly mating systems (Svärd & Wicklund 1989, Karlsson 1995). As the underlying biology of caddis flies and butterflies is so similar, many of these assumptions and subsequent predictions might be applicable to adult Trichoptera.

Mating systems in butterflies vary from strict monandry to strong polyandry (Svärd & Wiklund 1989, Gage 1994, Karlsson 1995), and mating system type affects the mating frequency of both sexes (Bissoondath & Wiklund 1996). In butterflies, as with many insects, males transfer nutrients to females at mating (Vahed 1998). In the case of butterflies these 'nuptial gifts' take the form of ejaculate material produced by male accessory glands, which is absorbed within the female reproductive tract. Female butterflies have been found to incorporate such material into their own body reserves and/or their eggs (e.g. Boggs 1981, Boggs & Watt 1981, Watanabe 1988, Wiklund *et al.* 1993, Svärd & McNeil 1994). Spermatophore size has a direct bearing on the refractory period of females (e.g. Oberhauser 1989, Wiklund & Kaitala 1995) and the last male to mate with a female usually fertilises her remaining eggs (Gage 1994). Sperm number has also been found to be important in fertilisation success under conditions of sperm competition (e.g. Parker 1970, Gage & Cook 1994). Hence there is a stronger selection pressure on males in polyandrous species to be able to produce several large ejaculates compared to males from monandrous species (Bisoondath & Wiklund 1996). Males of polyandrous butterfly species reflect this in an increased investment of larval reserves into reproduction (Karlson 1995), larger ejaculates (Svärd & Wiklund 1989, Karlsson 1995), higher nutrient content of reproductive reserves and ejaculates (Bissoondath & Wiklund 1995, Karlsson 1996) and greater ejaculate production capacity (Svärd & Wiklund 1989).

Boggs (1981) was the first to suggest that the resources invested in the abdomens of recently eclosed lepidopterans could be used as an approximation of reproductive reserves. Newly emerged individuals have empty guts and malphigian tubules, and so most of the abdominal content (haemolymph, fat body and reproductive organs) at emergence is primarily concerned with reproduction. Nitrogen is the most important of the abdominal resources, as it makes up a large

proportion of both eggs, and sperm/spermatophores (Marshall 1982), but it is also a limiting resource as adult lepidoptera often only feed on nectar, which has a very low nitrogen content (Baker & Baker 1973, 1986). The benefit of measuring abdominal reserves at eclosion, rather than just gonad size, is that the females of many species emerge with immature ovaries. This method of measuring reproductive potential can therefore be far more useful, as it allows comparison across species of the investment in reproduction at eclosion, regardless of ovarian developmental dynamics. Abdomen mass is also a good predictor of abdomen nitrogen content both within and between species (Karlsson 1995).

The amount of larval reserves allocated to reproduction at metamorphosis will therefore be dependent upon larval nutritional history, and expected adult intake (feeding and/or females receiving nutrients from mating) and output (female egg laying or male ejaculates) of nutrients. As such, individuals expecting an income of nutrients as adults generally invest less in their abdomens at eclosion than those with a limited adult intake. In other words, polyandrous females invest less larval reserves in reproduction than monandrous ones (Karlsson 1995). As males are investing more into reproduction with increasing female mating frequency, this leads to a general reduction in sexual size dimorphism (SSD) in reproductive allocation with increasing polyandry.

Those resources not invested into the abdomen will be invested mainly in the thorax. The thorax is approximately 95% flight muscle (Marden 1989, 2000) and so can be considered as an approximation of somatic investment (Karlsson & Wickman 1989). Nitrogen is again an important resource here because muscle is protein. Thorax size is, not surprisingly, correlated with flight ability in a number of flying insects. Species with high flight-muscle ratios (thorax mass: body mass) are faster and more manoeuvrable (Marden 1989, Srygley & Kingsolver 1998), which has obvious benefits in evading aerial predators. They are also able to lift heavier loads (Petersson 1995); in many species, one sex carries its mates whilst flying, and therefore high flight-muscle ratios will be important (Rutowski 1997). In swarming caddis flies males that successfully carried their mates to shore had larger flight muscles than those that failed, but the flight muscle ratio was not different between the two groups (Petersson 1995).

Many aspects of the life histories of adult butterflies are correlated with their morphology (i.e. pattern of resource investment), but the two relationships with the most relevance to caddis flies are those with mating systems and flight ability. The Trichoptera are the sister group of the Lepidoptera (Morse 1997) and as such share many aspects of their biology. Unlike the butterflies however, the details of the life history of the adult stage is poorly known. Adult caddis flies are generally small, drab, nocturnal and short lived, and this has obviously affected the amount of research conducted (Crichton 1957). Most information on adult ecology has come from light-trap records, but this has generally just been used to provide flight periods for males and females (Halat & Resh 1997). There has been little attempt to elucidate mating systems (Hoffmann 1999) or flight ability (Svensson 1974).

However, as the predictions, and consequently the observations on the relationship between resource allocation and life-history variables in the butterflies are likely to be applicable to caddis flies, this can help us in investigating caddis fly life histories. This means that even though some aspects of caddis fly adult biology have been difficult to observe in the wild, by using resource allocation patterns we can make suggestions concerning species life history variables. In this chapter, the morphologies of adult caddis flies from several species are analysed in an attempt to predict some of the unknown features of life history strategy. This will assist in the determination of patterns in resource allocation that may relate to adult ecology. The emphasis throughout this chapter is on SSD, mating system and flight ability.

7.2. METHODS

The details of data collection can be found in the methods sections of Chapters 4 and 5. Fore-wing length, and dry thorax and abdomen masses for 19 species of Limnephilid caddis flies were used in the analysis. Thorax and abdomen nitrogen content was also collected from five of these species and longevity data from six. Flight muscle and abdomen fat content was obtained for eight species. Data were obtained from specimens caught in a light trap during 1997, or from individuals reared in captivity during 1999. For species where data were obtained from the light trap, mean values for thorax and abdomen mass were obtained from individuals caught during the early part of the flight period, to account for any mass losses that can occur during the lifetime of individuals (see Chapter 5). Animals reared in captivity were frozen a few hours after emergence. This way the all values obtained were as close to what they would be at eclosion as possible. Structural body sizes such as wing length do not change over the course of an individuals lifetime, and so mean wing length values were calculated using all individuals. In some cases where no females of a species were collected, female wing lengths were obtained from Macan (1973). To use the abdomen as a meaningful predictor of reproductive investment, it needs to be expressed as a proportion of the reproductive investment by the other sex, in this case the female, as female abdomens are larger than males. The best way to predict flight ability is to measure the thoracic investment as a proportion of total investment in the body, which is mainly just the thorax and abdomen in caddis flies.

As there is no sub-family phylogeny for British Trichoptera (P. Barnard *pers. comm.*), it was not possible to do a comprehensive cross-species analysis controlling for the confounding effects of common ancestry (Harvey & Pagel 1991). An attempt at measuring polyandry directly by dissecting out the female reproductive system and counting the remains of spermatphores was unsuccessful. No clearly distinguishable, intact spermatophores were found after dissecting the females from four separate species that were caught in the light trap. To my knowledge, there are currently no reported measures of mating frequency in Limnephilids.

7.3. RESULTS & DISCUSSION

Wing lengths and dry thorax and abdomen masses for the 19 species are presented in Table 7.1. Female caddis flies are usually larger than males (Petersson 1995), as is the case with many insects (Darwin 1871). The species analysed here covered a range of body sizes, even just within the genus *Limnephilus* (Table 7.1). There is also a large variation in the male/female size ratio, in terms of both overall body size and reproductive investment. In *C. villosa,* female wing length is 18% longer than males (Table 7.2), whereas in *A .wallengreni*, female wing length is reported to be 13% shorter than males (no females caught in this study, so data are from Macan 1973). Male reproductive investment relative to that of females (male abdomen mass as a proportion of female abdomen mass) also varies (Table 7.2), from just 11% in *C. villosa* to 93% in *L. flavicornis*.

As body size increases, the degree of sexual size dimorphism (SSD) decreases such that large species are less dimorphic than small ones. In the closely related butterflies, such a shift in SSD is related to the degree of polyandry and relative male ejaculate size (Wiklund & Forsberg 1991). As the degree of polyandry increases, the value of male body size increases and that of female body size decreases (Karlsson *et al.* 1997), and as such males invest more in reproduction (Karlsson 1995). This may explain why the log of proportion of mass in the abdomen compared to the proportion in the thorax increases with log wing length in males ($r^2_{(adj)} = 15.5\%$, $p = 0.054$, df = 18; Fig. 7.1a) but there is no such significant relationship for females ($r^2_{(adj)} = 0.0\%$, $p = 0.692$, df = 13, Fig. 7.1b). Leimar *et al.* (1994) and Karlsson *et al.* (1997) suggest that nuptial gift giving occurs in conditions where juvenile food quality and/or quantity fluctuates, such that some females are potentially in need of resources and some males are in a position to provide. This situation is suggested to be important in the evolution of both polyandry and SSD. There is to my knowledge no data available on the range of adult caddis fly sizes, and the data set from this study is not large enough. Nevertheless, females of *L. lunatus* are recorded in Macan (1973) as having highly variable wing lengths, suggesting a high degree of varaiblity in larval food supply. This species is probably

Table 7.1 Wing lengths (mm) and thorax and abdomen dry masses (mg) for 19 species of Limenphilid caddis flies. Data shown are means with standard errors in parentheses. LT = Specimens recovered from a light trap, R = Specimens reared from pupae. *n* = sample size.

	n	Wing length	*n*	Thorax mass	Abdomen mass
Species					
Apatania wallengreni	19♂	8.09 (0.06)	20♂	0.81 (0.03)	0.31 (0.02)
Chaetopteryx villosa	8♂	8.51 (0.15)	8♂	1.35 (0.07)	0.85 (0.10)
	2♀	10.28 (0.23)	2♀	3.22 (0.09)	7.51 (1.73)
Glyphotaelius pellucidus	7♂	13.54 (0.20)	3♂	7.25 (0.94)	9.54 (0.92)
	14♀	13.79 (0.18)	3♀	8.43 (0.23)	12.51 (1.13)
Halesus digitatus	2♂	22.35 (0.75)	2♂	7.94 (0.31)	6.43 (1.27)
	1♀		1♀		
Halesus radiatus	55♂	18.35 (0.19)	26♂	5.18 (0.27)	4.14 (0.39)
	19♀	18.48 (0.43)	7♀	6.42 (0.53)	9.00 (1.76)
Limnephilus centralis	7♂	9.17 (0.22)	4♂	1.31 (0.14)	1.34 (0.21)
Limnephilus flavicornis	1♂		1♂		
	1♀		1♀		
Limnephilus griseus	2♂	9.75 (0.45)	2♂	1.97 (0.19)	1.17 (0.30)
Limnephilus lunatus	93♂	11.29 (0.07)	25♂	3.19 (0.19)	3.08 (0.25)
	40♀	11.72 (0.13)	6♀	3.21 (0.27)	4.45 (1.22)
Limnephilus luridus	5♂	10.56 (0.30)	1♂		
	1♀		1♀		
Limnephilus marmoratus	24♂	13.74 (0.17)	13♂	4.12 (0.33)	2.74 (0.50)
	9♀	14.50 (0.32)	5♀	6.89 (0.37)	8.06 (0.47)
Limnephilus nigriceps	1♂		1♂		
Limnephilus rhombicus	6♂	17.99 (0.33)	6♂	9.70 (0.60)	16.93 (1.17)
	8♀	17.72 (0.15)	8♀	11.32 (0.85)	18.14 (1.81)
Limnephilus sparsus	25♂	9.90 (0.18)	25♂	1.57 (0.11)	0.91 (0.10)
	7♀	10.40 (0.30)	8♀	2.29 (0.25)	3.42 (0.68)
Mesophylax impunctatus	3♂	14.65 (0.33)	3♂	3.35 (0.51)	1.54 (0.61)
	5♀	15.57 (0.75)	5♀	3.44 (0.26)	3.79 (0.85)
Micropterna lateralis	12♂	14.88 (0.27)	2♂	5.55 (0.58)	5.10 (0.88)
Potomophylax cingulatus	7♂	16.65 (0.25)	7♂	7.00 (0.40)	9.52 (0.68)
	6♂	16.25 (0.32)	6♂	7.06 (0.53)	14.46 (1.92)
Potomophylax latipennis	1♂		1♂		
	3♀	16.75 (0.28)	3♀	5.04 (0.71)	3.82 (1.15)
Stenophylax vibex	9♂	20.66 (0.31)	9♂	6.27 (0.40)	7.11 (1.29)
	1♀		1♀		

Table 7.2 Relative male body size, reproductive investment and somatic investment, resource and ovarian dynamics, and the predicted mating systems for 15 species of Limnephilid caddis flies. Relative body size is mean male wing length expressed as a percentage of mean female wing length. Relative reproductive investment is the mean male abdomen mass expressed as a percentage of mean female abdomen mass. Relative somatic investment is mean male thorax mass expressed as a percentage of mean female thorax mass. Mass loss refers to whether there is a significant loss of mass from the thorax (TH) or abdomen (AB) during individuals lifetime (data from Chapter 5, Table 5.3). The presence or absence of an ovarian diapause is taken from Svensson (1972). Species are ranked by relative male reproductive investment, and this is used to predict the mating system, which is assumed to be a continuum from polyandry to monandry. *Apatania wallengreni* is placed last because its mating system is predicted to be very different to the others (see Results and Discussion).

Table 7.2

Species	Male body size (relative to female)[1]	Male reproductive investment (relative to female)	Male somatic investment (relative to females)	Mass losses	Ovarian diapause	Predicted mating system
Limnephilus rhombicus	102%	93 %	86%	-	YES	POLYANDRY
Glyphotaelius pellucidus	**100%**	76 %	86%	♂ TH & AB	YES	POLYANDRY
Limnephilus lunatus	**93-127%**	69 %	99%	♂ TH & AB	YES	POLYANDRY
Potomophylax cingulatus	102%	66 %	99%	-	NO	POLYANDRY
Stenophylax vibex	**104%**	57 %	98%	-	YES	
Potomophylax latipennis	**101%**	54 %	81%	-	NO	
Limnephilus flavicornis	100%	47 %	116%	-	YES	
Halesus radiatus	99%	46 %	81%	♀ TH	NO	
Halesus digitatus	102%	42%	95%	-	NO	
Mesophylax impunctatus	94%	41 %	97%	-	NO	
Limnephilus sparsus	95%	35 %	81%	-	YES	MONANDRY
Limnephilus marmoratus	95%	34 %	60%	♀ TH	YES	MONANDRY
Limnephilus luridus	86%	30 %	83%	-	YES	MONANDRY
Chaetopteryx villosa	82%	11 %	42%	-	NO	MONANDRY
Apatania wallengreni	113%	-	-	♂ AB	NO	(SWARMING)

[1] Figures in bold represent data taken from Macan (1973)

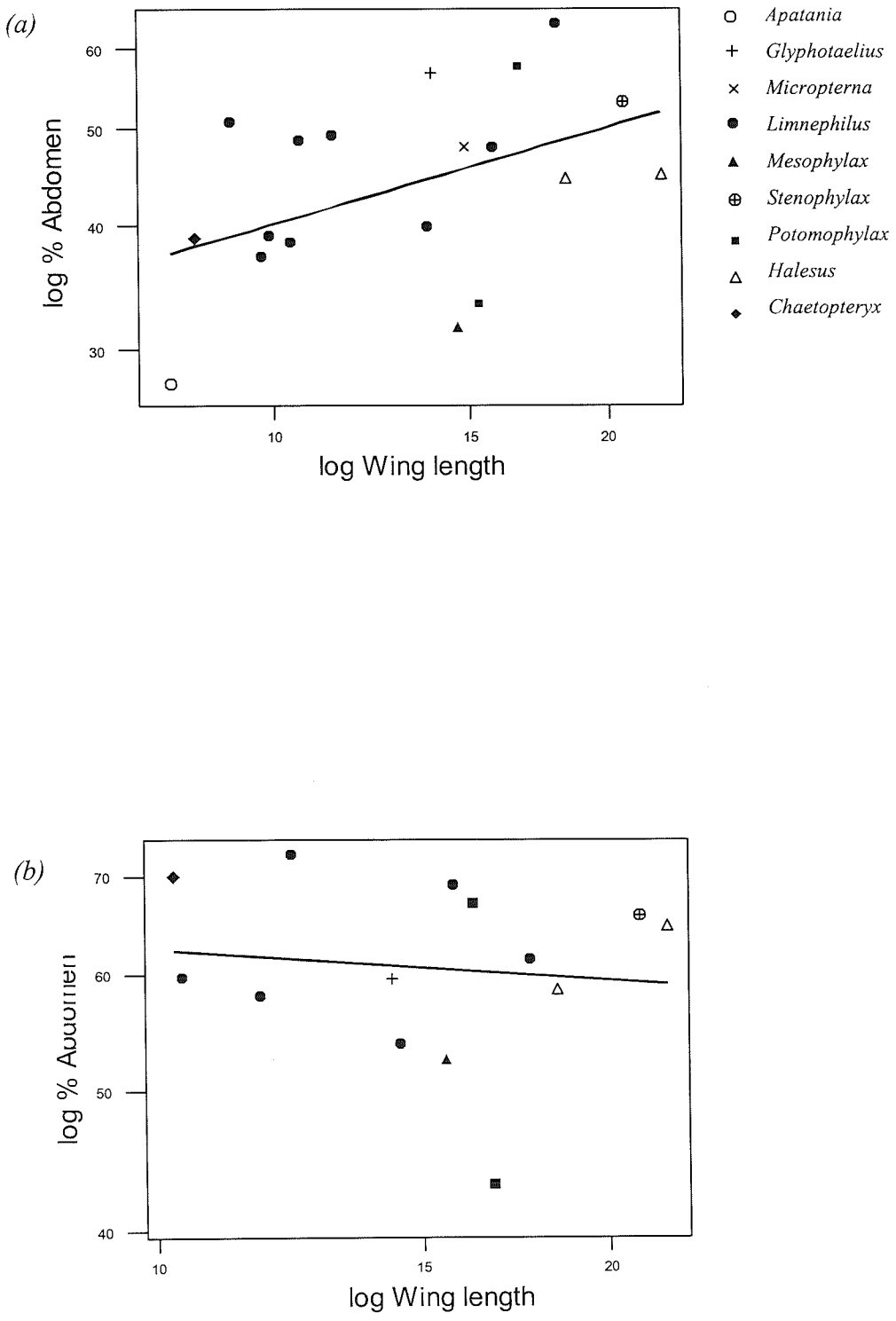

Fig. 7.1 The relationship between wing length and the proportion of mass in the abdomen compared to the thorax across genera divided into (a) male and (b) female Limnephilid caddis flies. For (a) there are 19 species within nine genera, and for (b) there are 14 species within seven genera.

Table 7.3 Percentage nitrogen content at eclosion of adult thoraces and abdomens from five species of Limnephilid caddis flies. Data shown are means with standard errors in paraentheses. *n* = sample size.

	n	Thorax nitrogen %	Abdomen nitrogen %
Glyphotaelius pellucidus	11♂	12.09 (0.19)	11.68 (0.35)
	13♀	11.91 (0.18)	10.74 (0.19)
Limnephilus flavicornis	2♂	9.00 (2.33)	10.34 (2.01)
	2♀	11.93 (0.22)	10.92 (0.21)
Limnephilus marmoratus	1♂	11.49	7.69
	2♀	12.23 (0.94)	8.13 (0.55)
Limnephilus rhombicus	3♂	11.50 (0.34)	11.15 (0.69)
	4♀	10.57 (0.90)	8.06 (1.13)
Potomophylax cingulatus	5♂	9.70 (0.96)	11.72 (1.29)
	3♀	11.99 (0.52)	11.58 (0.28)

Table 7.4 The relative mass of adult thoraces at eclosion for 19 species of caddis flies. Values are shown as the percentage of thorax and abdomen mass within the thorax. Data are means with standard errors in parentheses. n = sample size.

		% Thorax mass	Predicted flight ability
Species			
Apatania wallengreni	20♂	72.39 (1.15)	STRONG
Chaetopteryx villosa	8♂	61.84 (1.38)	STRONG
	2♀	30.83 (5.52)	WEAK
Glyphotaelius pellucidus	3♂	42.98 (1.48)	
	3♀	40.46 (1.73)	
Halesus digitatus	2♂	55.68 (3.97)	
	1♀	35.23	
Halesus radiatus	26♂	57.35 (1.64)	
	7♀	44.31 (3.52)	
Limnephilus centralis	4♂	49.77 (3.06)	
Limnephilus flavicornis	1♂	52.20	
	1♀	30.82	WEAK
Limnephilus griseus	2♂	63.43 (3.86)	STRONG
Limnephilus lunatus	25♂	52.06 (1.73)	
	6♀	47.34 (6.86)	
Limnephilus luridus	1♂	51.45	
	1♀	27.48	WEAK
Limnephilus marmoratus	13♂	62.69 (2.82)	STRONG
	5♀	46.16 (1.76)	
Limnephilus nigriceps	1♂	61.54	STRONG
Limnephilus rhombicus	6♂	36.49 (0.81)	
	8♀	39.04 (2.65)	
Limnephilus sparsus	25♂	64.95 (1.65)	STRONG
	7♀	42.61 (2.53)	
Mesophylax impunctatus	3♂	70.55 (4.97)	STRONG
	5♀	50.04 (5.64)	
Micropterna lateralis	12♂	57.08 (1.71)	
Potomophylax cingulatus	7♂	42.55 (1.80)	
	6♀	33.65 (2.33)	WEAK
Potomophylax latipennis	1♂	66.65	
	3♀	58.54 (3.28)	
Stenophylax vibex	9♂	49.77 (3.28)	
	1♀	33.98	WEAK

polyandrous. This is one area warranting further investigation, in both butterflies and caddis flies.

With a lack of any data on mating frequency in Limnephilid caddis flies, I propose the measurements of relative reproductive investment reported here to be good indicators of caddis fly mating systems (Table 7.2). Species where males invest a large proportion of mass in the abdomen relative to the equivalent female investment are suggested to be polyandrous, based on similar patterns in butterflies. The sexual dimorphism in abdomen mass is not reflected in wing length or thoracic mass, which may also indicate how strongly abdomen investment is related to mating system. One potentially confounding factor is the ovarian diapause undergone by the females of many Limenphilid species (Novak & Sehnal 1963). Rutowski (1997) suggests that female reproductive investment at eclosion may be influenced by ovarian development in butterflies, with those developing eggs during adult life having smaller abdomens at eclosion. However no such relationship was seen here (Table 7.2).

The predictions on degree of polyandry based on the comparison of male and female reproductive investment is corroborated by the relative investment of abdominal nitrogen and also to some extent data on patterns of mass loss (Chapter 5). High relative male investment in abdomen mass by *L. rhombicus* and *G. pellucidus* was mirrored by males having a significantly higher percentage of nitrogen in their abdomens than females (Table 7.3) (*L. rhombicus* $F_{1,6} = 16.72$, $p = 0.015$; *G. pellucidus* $F_{1,23} = 10.48$, $p = 0.004$) . Males of *L. marmoratus*, where male relative abdomen mass is small, had a lower proportion of nitrogen in their abdomens than conspecific females, although small sample sizes prevent statistical testing of this. One feature of polyandrous butterfly species is that males have increased nutrient content of reproductive reserves (Bissoondath & Wiklund 1995, 1996, Karlsson 1996). The results from this study on caddis flies would suggest that patterns of investment into reproductive reserves, of both mass and nitrogen, indicate that some species are more polyandrous than others. The decline in mass of the thorax that occurs during the lifetime of some nectar-feeding butterflies is also correlated with the type of mating system (Karlsson 1994, 1998, Stjernholm & Karlsson 2000). Females from the few species that have been studied seem to histolyse their flight

muscles to provide nitrogen for reproduction, whilst this only happens in males from polyandrous species (Stjernholm & Karlsson 2000). In Chapter 5, thoracic mass loss was reported for males from two species (*G. pellucidus* and *L. lunatus*) whose reproductive investment relative to that of the females indicates polyandry.

Relative thorax mass is known to be a predictor of flight ability in butterflies (e.g. Dudley & Srygely 1994), as it is highly correlated with flight muscle content (Marden 1989). In this study (Table 7.3), male *A. wallengreni* had a very high investment in thoracic mass compared to abdominal mass (72%). Male wing span was also much longer in males than females, and these two facts together would suggest that this species forms mating swarms, where the male carries the female in flight (Petersson 1995). Gullefors & Petersson (1993) found that when the size ratio of male/female Leptocerids is greater than 1.0 (as it is in *A. wallengreni*), then coupling of males and females occurs in flight, as opposed to on the ground or on vegetation. Data from this study would suggest that *A. wallengreni* is a swarming species, with copula formation occurring in flight. Swarming is an unusual characteristic of Limnephilids (Ivanov 1991). Wing shape of this species (*pers. obs.*) is also characteristic of improved flight ability (Ross 1967). Both sexes of *M. impunctatus* and *P. latipennis* had relatively high thoracic investment, but are unlikely to be species where mate formation occurs in swarms because they are too large. Not enough is known about the ecology of these species to determine why thoracic investment is so high. It may be due to high mate search costs (Wickman 1992) or predator avoidance (Srygley & Kingsolver 1998). It may even be related to thermoregulatory behaviours (Van Dyck *et al.* 1998) as *Potomophylax* has been reported to be able to fly in night time temperatures below 5^{o} C (Solem 1983). Of the other species, there was a range of thorax masses, but male potential flight ability was almost always higher, as is the case in butterflies (Karlson & Wickman 1989). Quantifying caddis fly flight behaviour has proved very difficult in the laboratory though (*pers. obs.*, J. Gee *pers. comm.*), and light trapping does not give enough information (Svennson 1974). More sophisticated field studies may be the only way of measuring adult flight performance.

The morphological correlates in this study provide a framework of predictions that could be tested in the laboratory (e.g. mating systems) or with more

difficulty, in the field (e.g. flight ability). Despite their similarities, potential difference in the life style of caddis flies, for instance their long larval period, high larval silk expenditure (Chapter 2 & 3) or their nocturnal adults, could provide additional selective pressures on resource allocation patterns. These might potentially change the relationships between morphology and life-history. For instance, in nocturnal moths the relationship between flight morphology, tympanic hearing organs (related to evasion of bat predators) and thermoregulation (Rydell & Lancaster 2000) may be more relevant to caddis flies than some of the work on diurnal butterflies. One interesting difference between butterflies and caddis flies is that butterflies produce apyrene sperm (small, anucleate sperm), whereas this is has not been found in the species of caddis flies where it has been looked for (Friedländer 1983). Apyrene sperm have been implicated in sperm competition, but the exact function of it is unkown (e.g. Silberglied *et al.* 1984, Gage 1998, Cook & Weddell 1999, Morrow & Gage 2000).

The presence or not of apyrene sperm definitely warrants further study, as it may be an indicator of differences in the order that mating and oviposition occur in, when caddis flies and butterflies are compared. The relationship between mating system and resource allocation may in fact be very different to that found in Lepidoptera, despite the two orders being so closely related (Morse 1997). For instance, if spermatophore size in caddis flies has evolved to provide nutrients to females, then the increased assurances of paternity in monogamous systems, would select for less male investment in reproduction under polyandry, rather than more (Gwynne 1984, Morrow & Gage 2000). If this were the case the relationship between relative reproductive investment and mating system suggested here, would be completely reversed.

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Chapter 8. General Discussion

There are many relevant aspects of developmental trade-offs that fall outside the scope of this study, however an attempt has been made to cover the main factors that may affect resource allocation patterns in insects with complex life cycles. In the following discussion, I aim to link these together and suggest future work that could arise from this study.

It is well known that the resource allocation patterns arising during the development and growth of living organisms will be constrained to some extent by their evolutionary history (Harvey & Pagel 1991) and also through selection acting upon factors that influence fitness (Roff 1992, Stearns 1992). What has been harder to demonstrate is the extent to which limited resources during development lead to trade-offs between body parts and how this might regulate the relative sizes of organs (Trumbo 1999). Holometabolous insects are proving good model systems to investigate both the forces shaping resource allocation patterns during development, and how these forces themselves interact.

Various factors operating during the larval stage of such insects will determine the type and the quantity of resources with which an individual enters into pupation with. These factors will include the quality of diet (e.g. Tammaru 1998), and the time spent foraging (e.g. Gotthard 2000), both of which will have obvious affects on resource accrual, and thereby on growth rate. High temperatures during the larval stage are known to increase development rate (e.g. Sibly & Atkinson 1994) and time constrained (e.g. late season) individuals also develop at a faster rate (e.g. Johansson & Rowe 1999). Both growth rate and development rate of larvae will heavily determine the size and amount of specific resources an individual has available to build the adult body form. Expenditure of resources by larvae, such as through noxious secretions (Grill & Moore 1998), sugar-feeding of mutualistic ants (Bayliss & Pierce 1992) or silk spinning (Dudgeon 1987), will also affect the resource budget at metamorphosis.

Re-allocation of larval derived nutrients into adult tissues will, as mentioned above, to some extent be determined by phylogenetic constraints and also adjusted to the life history of the adult form. It is potentially flexible within these limits however, with allocation to certain structures gaining priority when specific nutrients are limiting.

Diet deficiencies in caterpillars of *Parage aegeria* were found to lead to smaller individuals overall, but proportional allocation to thoraces and abdomens changed (Karlsson *et al.* 1997). Similarly, a low protein diet in male moth larvae (*Plodia interpunctella*) led to a change in sperm numbers but not sizes (Gage & Cook 1994). Juvenile cockroaches provided with low food quantities preferentially allocated hydrocarbons to waterproofing the adult cuticle rather than internal organs (Young *et al.* 1999). In this study, increased silk expenditure by larval caddis flies leads to adults with lower investment in reproductive allocation (Chapter 2) or somatic investment (Chapter 3). In all these cases, the life history requirements of the adult form have been proposed to be a major factor in determining which tissues gain precedence in allocation over other structures, when nutrients are limiting.

With no growth in the adult stage, and a diet that is usually very limited in nutrients (Baker & Baker 1973, 1986), the way resources are allocated at emergence will have very profound affects on fitness (Boggs 1981). Whilst this is true, recent evidence suggests that organisms such as butterflies and caddis flies have a more dynamic allocation strategy than once thought (Karlsson 1994, 1998; Stjernholm & Karlsson 2000, Chapter 5). Without a source of nitrogen in the diet, muscle tissue cannot be added to after emergence (Karlsson 1994), but it can be broken down and the liberated nitrogenous compounds incorporated into reproductive tissues (Stjernholm & Karlsson 2000). This means that even within a life history stage, an organism's changing resource-needs can be addressed. Flexibility in allocation may also to some extent cushion the effects of any nutrient deficiencies that may have occurred during the larval stage. However, whether such compensatory measures later in life have costs attached, also remains unknown (Metcalfe & Monaghan in press). Much more work is needed to bring about a better understanding of the fitness consequences of particular allocation decisions.

The benefit of moving nitrogen between somatic and reproductive functions in the adult stage will, however, probably be dependent upon the ability to produce specific proteins, such as vitellogenin, from this thorax-derived nitrogen. However, this will be dependent upon the amino acids available in the adult stage. Amino acids are very important in the biology of adult butterflies and caddis flies, because of their almost total absence from the diet (Baker & Baker 1973, 1986) and the significant

proportion they make up of muscle, eggs and spermatophores. However, the larval stage is often herbivorous which can be protein poor (Scriber & Slansky 1981), and many species of both the Trichoptera and Lepidoptera produce large quantities of silk (Craig 1997) which is largely composed of protein. Silk costs mainly depend on the percentage of the silk consisting of ingested amino acids, opposed to amino acids that have to be synthesised, which requires energy (Craig *et al.* 1999). Specific amino acids may therefore be expended in large quantities in the larval stage, through silk use. Excessive silk production will lead to a decrease at pupation, not just of total nitrogen, but of specific amino acids. As the adult diet will be severely lacking in amino acids (Baker & Baker 1973, 1986), these amino acids may have to be synthesised if required for the adult stage. The amount of essential amino acid residues in silk will also be very important, as these cannot be synthesised, and are therefore only available from the larval diet. Craig *et al.* (1999) suggest that the differences in the amino acid composition between spider and Lepidopteran silks is related to the amino acid and energy composition of their respective diets.

Nitrogen is often used to determine resource allocation patterns within insects (Boggs 1981, Karlsson 1995, Stjernholm & Karlsson 2000, Marden 2000). However, it is more specifically amino acids that should be measured, as they vary in abundance within both larval and adult diets, and arthropods can synthesise only half of the 20 amino acids they need (Craig *et al.* 1999). Holometabolous insects carry amino acids through pupation within hexameric storage proteins (Telfer and Kunkel 1991). These proteins are found within the fat body, and are broken down for the production of the adult during metamorphosis, but many hexamerins may also last some time into the adult stage, for instance to provide amino acids for oogenesis (Wheeler & Martinez 1995, Wheeler & Buck 1996, Wheeler *et al.* 2000). With regard to this study, the amino acid contents of caddis fly silk and adult tissues would be very interesting to examine, as would the form nitrogen is in when transferred from thorax to abdomen in adults. The difference responses of two caddis fly species to the same manipulation of silk expenditure, may be related in part to the amino acid content of their silks and adult tissues. Equally, the movement of nitrogenous compound within the adult may or may not occur due to the availability and requirements of particular amino acids.

One other feature of the nitrogen budget of pupae and adults that is worth investigating, is that of protein turnover. Protein turnover is the balance between synthesis and degradation, and both processes are energetically expensive (Houlihan 1991). For instance, if particular amino acids were lacking within the pupae or adult, perhaps as a result of increased silk expenditure, certain adult-tissue proteins may have to be continually broken down so as to release the specific amino acid to build other proteins. These may in turn be broken down to supply construction of the original protein. This continual turnover to compensate for deficiencies in the amino acid pool would be energetically expensive. This may explain the why there was a mass loss from thoraces (*O. Albicorne*) and abdomens (*G. Pellucidus*) greater than just the loss of protein mass from these tissues. In fact overall mass loss was proportionally higher than nitrogen loss, such that proportional nitrogen content was (non significantly) higher within the tissues of rebuilding animals. If it was an energetic expense from extra larval case building, we might expect a more generalised affect on adult size, rather than an organ specific response.

As well as a more specific analysis of nitrogen dynamics across the life cycle, more work is obviously required into the life history traits of adult caddis flies. In Chapter 7, the mating systems and flight ability of a number of species were predicted from their patterns of mass allocation. These testable associations would hopefully provide information, not just on caddis flies, but also to the generality of the theories originally formulated for butterflies. Analysis of adult caddis fly resource allocation decisions and their fitness consequences is difficult, but possible with a systematic, phylogenetically controlled approach, and large sample sizes. The extent to which Trichoptera differ from Lepidoptera (e.g. Long larval period, long adult life span, nocturnal flight) and how this alters the relationship between general body size & shape and life history will be interesting, particularly with respect to their close phylogenetic association. It will also have implications for the moths, which are another group whose resource allocation patterns have received little attention, for similar reasons to caddis flies. A real breakthrough in resource budget analysis would be to determine the extent of adult feeding and the source of food utilised. The degree to which larval resource manipulations, and the subsequent affects on adults, bear on fitness traits is also of great interest, yet is poorly understood (Trumbo 1999).

Potentially though, the absence of apyrene (anucleate) sperm in Trichoptera could be the most important difference between the groups. Although its exact function is unknown (Morrow & Gage 2000), apyrene sperm has been implicated in sperm competition. A lack of apyrene sperm may indicate that alternative mechanisms are employed by males to ensure fertilisation success. It may alternatively indicate that females alternate matings with oviposition (sperm- replenishment polyandry, Thornhill & Alcock 1983) such that direct sperm competition does not take place. This is however unlikely given the presence of spermatophores, (material benefit polyandry –Thornhill & Alcock 1983) at least within the Limnephilidae. A first step would be to analyse spermatophores from different species, in terms of sperm content and also nutrient content. Males transfer a range of nutrients to females at mating in butterflies, including protein, sodium, hormones and lipids (Marshall 1982, Bissoondath & Wiklund 1995), and this may well be the case in caddis flies. This in turn could be related to the nutrients obtained in the adult diet, and those provisioned to the eggs.

Animals with complex life cycles, where the body form and the habitat can change dramatically during ontogeny, provide good model organisms for investigations into the connections between resource allocation patterns, development and life history. The Trichoptera and Lepidoptera in particular have proved very useful, as pupal resource levels can be easily altered through manipulations of resource income (e.g Gage 1995, Karlsson *et al* 1997), or resource expenditure (e.g. Bayliss & Pierce 1992, Chapter 2 & 3). The diet of the larval and adult stage differ markedly, such that certain resources gain a greater importance than others. Finally, resource allocation is easily measured as it is very obviously expressed through adult morphology, and it provides a good indication of adult life history traits.

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